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GLOSSARY OF TERMS USED IN BIOMOLECULAR SCREENING

(IUPAC Recommendations 200?)

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(IUPAC Recommendations 2008)

Abstract: Biomolecular Screening is now a crucial component of the drug discovery process and this Glossary of Terms will be of use to practitioners in the field of screening and to those who interact with the screening community. The glossary contains definitions related to various aspects of the screening process such as assay types, data handling and relevant technologies. Many of the terms used in this discipline are not covered by existing glossaries, and in the cases they are, the definitions are often not appropriate for this field. This document provides new or modified definitions to better reflect the new context. The field of Biomolecular Screening is multidisciplinary in nature and this glossary containing authoritative definitions will be useful not only for regular practitioners, but also for those who make use of the data generated during the screening process.

1 absorbance assay
Obsolete term: optical density assay
Assay in which response is determined by the detection of light absorption by an assay component. The concentration of the absorbing species can be quantified using the Beer-Lambert Law (or Beer-Lambert-Bouguer Law)

$$A = \varepsilon c l$$

where ε is the molar absorption coefficient of the absorbing species, l is the absorption pathlength, and c the concentration. A sample is irradiated with a specific wavelength of light, typically of the ultraviolet or visible spectrum; the spectral radiant power of incident light on the sample (P_o) and transmitted radiation after passing through the sample (P), are used to calculate absorbance (A). [1]

$$A = \log_{10}\left[\frac{P_0}{P}\right]$$

2 accuracy
Closeness of the agreement between the result of a measurement and a true value of the property being measured.[2]

3 active n, adj
Sample that produces a response or signal above a defined threshold at a tested concentration in a single *assay* or *screen* and that has not yet been confirmed by subsequent experiment.
Note 1: When the properties and identity of an *active* are confirmed by subsequent experiment it becomes a *hit*.
Note 2: Use of *active* and *hit* as synonyms is inaccurate.
Related term: *inactive*

4 activity
Response to a test sample measured in an *assay* or *screen*.
Note: Typically expressed as a percentage.

5 activity distribution
Plot or graphical representation of the number of samples present in each *activity* range.
Note: Often shown as a population bar chart, it provides an overview of the screening results and typically allows the determination of the overall *background* signal and threshold for selection of *actives*.

6 affinity
Descriptive, non-quantitative term that indicates the relative tendency of one molecule to associate or interact with another.
Note: Often misused to refer to the dissociation equilibrium constant, K_d measured for a *ligand*, or K_i for an *inhibitor*.
Related term: *potency*

7 agonist
Endogenous substance or drug that can interact with a receptor and initiate a physiological or a pharmacological response characteristic of that receptor (contraction, relaxation, secretion, enzyme activation, etc.).[3]
Note: A full agonist induces maximal *receptor* response for the biological system in question, while partial agonist does not.
Related terms: *partial agonist*, *inverse agonist*, *antagonist*.

8 allosteric site
Binding site on a protein, distinct from the site recognized by an endogenous agonist (i.e the *orthosteric site*) in the case of a receptor or substrate in the case of an enzyme, that when occupied by a ligand effects a conformational change in the protein thus altering its signaling or catalytic properties..

9 antagonist
Drug or compound that opposes the physiological effects of another. At the receptor level, it is a chemical entity that opposes the receptor-associated responses normally induced by another bioactive agent.[3]
Note: This term does not imply a specific mechanism which can include competitive, allosteric, non-competitive, or physiological. [4]

10 artifact
Experimental result that is not a manifestation of the phenomenon under investigation, but is brought about erroneously by the particular arrangement of instrument and method or by a random disturbance of the experiment. [5]
Related Terms: *false positive*, *false negative*.

11 assay
Experimentally controlled biochemical or biological system used for the quantitative analysis of perturbations imposed by a test sample.

12 assay validation
Experiments conducted to verify that the output measurement of the assay is reflective of the activity against the *target*. Results are compared internally over multiple runs and externally (when available) to existing literature parameters such as *Kd*, *Ki*, *Km*, or EC_{50}.

12 automated device
Synonym: automated peripheral
Device that performs one or more functions without human intervention by means of direct control or by programmed operations.
Note: Often referred to as a peripheral or peripheral device when it is an instrument incorporated into a module, a workstation or a fully automated system.

Examples: *liquid handler*, *module*, *workstation*

13 automation
Mechanization with process control, where process means a sequence of manipulations. One or several functions in an instrument may be automated.[2]

14 average
Arithmetic mean of a set of values. A non-technical term.

15 background
1. Amount of a signal produced in an assay or screen in the absence of a test substance.
2. Signal detected from an assay in the absence of *target* activity; often equivalent to negative control
Related terms: *negative assay control, noise*

16 batch
Homogeneous preparation of a reagent or reactant (small molecule, enzyme, *clone*, etc.) produced (synthesized, purified, or otherwise) at one time.

17 binding assay
Assay in which the specific interaction between two molecules (e.g. ligand-receptor, antibody-antigen, protein-protein, ligand-transport protein) is measured.
Note: The assay can be homogeneous or heterogeneous, competitive or non-competitive and may be run at equilibrium or in kinetic mode. Appropriate assay controls and/or standard reagents are often needed to determine the specific binding in contrast to non-specific adsorption processes.

18 B_{max}
Total number of receptors or binding sites in a system, $[N]_T$, as determined by analysis of a binding isotherm.
When the system is a simple one containing a single binding site and lacking cooperative interactions, binding is described by the following equation:

$$B = (B_{max}[L]) / (K_d + [L])$$

where B is the amount of ligand specifically bound, [L] is the free concentration of ligand, and K_d is the equilibrium dissociation constant of the ligand.
The equation describes a hyperbola with B_{max} as the asymptote. Units of B_{max} are amount of ligand bound per amount of binding material e.g. $pmol.mg^{-1}$.

19 cell-based assay
Assay in which the response of live cells to a test sample is measured. The type of cells, parameters measured, and detection systems used vary widely.

Related terms: *cytotoxicity*, *chemotaxis assay*.

20 cell membrane preparation

Preparation from tissue or cells, in which the membranous elements, generally the cell membrane, are enriched in relation to other cellular constituents. These are obtained by disruption of the cells and differential centrifugation or other techniques to isolate the desired components.

Note: Cell membrane preparations are the standard biological material for *receptor* binding assays.

21 cell mortality

Ratio of counted dead cells to the total number of observed cells. Dead cells are often counted using apoptosis or nuclear markers.

22 cell viability

Ratio of counted live cells to the total number of observed cells. Live cells are often counted using nuclear or membrane markers.

23 channel blocker

Compound that reduces or eliminates the conductance of an ion channel by impeding the movement of ions through that channel.

Example: Verapamil, which blocks the conductance of some L-type calcium channels, is an example of a channel blocker.

Note: Dihydropyridine antagonists such as nifedipine which also block the conductance of some L-type calcium channels, are not classified as channel blockers since their action is to inhibit channel gating. [6]

24 channel opener

synonym: channel activator

Compound that increases the conductance of an ion channel by a mechanism other than alteration of membrane potential or activation of the cognate receptor.

Examples: Retigabine and cromakalim.

25 cheminformatics

Use of mathematical and statistical methods to extract information from chemical data. [7]

Example: For screening purposes, cheminformatics approaches are used in order to design compound libraries for general screening or for screening against a specific target class.

26 chemotaxis assay

Cell based assay in which the rate and / or direction of cell migration is measured. The response to a test sample is typically detected by means of a fluorescent probe or with label-free methods.

27 clone

1. Population of cells derived from a single ancestor by asexual reproduction;
2. Exact copy of a DNA fragment, produced on a plasmid by recombinant DNA technology.

28 coefficient of variation (CV)

Standard deviation expressed as a percent of the mean.
CV = 100(standard deviation / mean).

29 combinatorial library

Set of compounds prepared by combinatorial chemistry. It may consist of a collection of pools, or sub-libraries. Its composition may be described by the chemset notation. [8]

30 competitive binding assay

Molecular assay based on the competition between a ligand and a reference ligand for the same binding site on a receptor (e.g. antibody, transport protein).
Depending on the technology used to monitor the interaction, the reference ligand and/or the receptor can be labeled with a probe. Very rarely, and mostly out of the field of screening, neither is labeled and the interaction is assessed, for example, by mass determination of the complex.
Note: Former definition of competition between labeled and non-labeled ligands is obsolete. [9]

31 compound collection

synonym compound library
Set of chemicals that has been assembled and annotated for easy storage and retrieval and that is available for screening.
Generally consists of compounds synthesized by combinatorial or standard synthetic methods, purchased from commercial sources or of samples of natural products either as pure samples or as mixtures.

32 concentration response relationship

Figure quantifying the effect of a compound in an assay at increasing concentrations.
Note: This experiment provides information about the potency and efficacy of the tested compound. Classical thermodynamics of a 1:1 interaction between compound and target generally results in a hyperbolic increase of the assay signal upon linear increase in compound concentration. Thus, compound concentration responses are usually determined using logarithmic serial dilutions, e.g. 10 µmol/L, 1 µmol/L, 100 nmol/L, 10nmol/L, 1 nmol/L.
Related terms: K_i, K_D, EC_{50}, IC_{50}.

33 counter-screen
Screen in which test samples are assessed against a *target* for unwanted activity. This *target* may or may not be structurally or functionally related to the intended target.

34 cytotoxicity assay
Cell based assay in which response to a test sample is determined by measuring cell death or *cell viability*.

35 EC_{50} (Effective concentration 50):
Concentration of a reagent that produces one half of the maximal response for that reagent in an assay.
Usually refers to an agonist in a receptor system.
Note: The effect could represent either an increase or a decrease in a biological function.
Related term: IC_{50}

36 efficacy
Extent to which a compound activates a receptor to produce a response in an assay under saturating conditions. This is usually compared to results with the positive and negative assay controls.
Note: When the compound produces a maximal signal that is 100% of that of the positive control, it is said to be a full agonist and has high efficacy. When the effect plateaus with increasing concentration and reaches an intermediary level of activity, the compound is said to be a partial agonist with lower efficacy. Due to the common overexpression of receptors in screening assays, it is not always possible to detect differences in efficacy among full agonists. A more accurate assessment of relative efficacy may require systems with lower receptor expression where it is often found that one agonist may show partial agonist character.
Related term: *partial agonist*

37 electrochemiluminescence assay (ECL)
Assay in which light emitted by an electrochemical reaction is detected and used to quantify a *probe*, ruthenium (II) tris-(bipyridine), on one of the two assay binding components.
Example: Coated magnetic beads bind the protein target and are then captured in a flow cell of the detector where the energy state of the Ru is chemically converted to release a photon measured at 620 nm with a photomultiplier tube. There is little or no compound interference during detection because of the wash step within the flow cell. Generally not used for HTS but for secondary assays

38 end-point assay
Kinetic assay run for a set constant incubation time. Typically the set incubation time is followed by the addition of a reagent that stops the reaction and allows postponed measurement of the signal.

Related terms: *equilibrium assay*, *kinetic assay*.

39 enzymes
Macromolecules, mostly of protein nature, that function as (bio)catalysts by increasing the reaction rates. In general, an enzyme catalyses only one reaction type (reaction specificity) and operates on only one type of substrate (substrate specificity). Substrate molecules are attacked at the same site (regiospecificity) and only one or preferentially one of the enantiomers of chiral substrates or of racemic mixtures is attacked (stereospecificity). [2]

40 enzyme-linked immunosorbent assay (ELISA or EIA)
Heterogeneous assay in which an antibody linked to an enzyme is used to detect the quantity of antigen present in a sample.
After binding of the enzyme-linked antibody to the antigen, either directly or indirectly via a second antibody, a subsequent reaction of the enzyme with a substrate yields a chromogenic or fluorogenic product that produces an amplified signal proportional to the concentration of the antigen.

41 epitope
synonym: antigenic determinant
Minimum molecular structure in an antigen that will react with an antibody. It may comprise only a portion of an antigen. [10][11]

42 epitope mapping
Identification and localization of the specific regions of protein molecules that are recognized by the immune system.

43 equilibrium assay
Assay in which there is sufficient incubation time for the plateau phase of the signal to be reached and equilibrium has been established between the reactants. At this point, the signal is time-independent.
Related terms: *kinetic assay*, *end-point assay*.

44 equilibrium association constant, K_a
synonym: K_{ass}
Ratio, at equilibrium, of [AB]/[A][B] for the reversible binding interaction of A and B to yield the complex AB. Units are M^{-1}.
Note: Equal to $1/K_d$, or k_{on}/k_{off}.

45 equilibrium dissociation constant, K_d
synonym: K_{diss}
Ratio, at equilibrium, of [A][B]/[AB], for the reversible binding interaction of A and B to yield the complex AB. Units are *M* (molar).

Note: Reciprocal of K_a, ($K_d = 1/K_a$), equal to k_{off} / k_{on}.
Related term: *inhibition constant,* K_i

46 false negative
Assay result in which a sample known to be active does not produce either the expected signal or a signal above the activity threshold.
Note: A false negative can occur when an assay lacks appropriate discriminatory power, when the threshold is inappropriately set, or as a result of mistaken identity of the test sample.
Related terms: *artifact, false positive*.

47 false positive
Assay result in which a sample known to be inactive produces a signal or response above the activity threshold.
Note: A false positive can occur when an assay lacks appropriate discriminatory power, when the threshold is inappropriately set, as a result of certain physical properties of the substance (e.g. a fluorescent compound in a fluorescence intensity assay, aggregation), or as a result of mistaken identity of the substance
Related terms: *artifact, false negative*.

48 flow cytometry assay
C*ell based assay* in which response is dependant on the detection of the phenotype of cells as determined through the light scattering properties of each cell, and, typically, the fluorescence intensity of a relevant *probe*.
Example: In fluorescence activated cell sorting (FACS), single cells are rapidly passed through a channel where they are optically analyzed and are separated based on response for further study.

49 flow injection (flow analysis)
Analytical methods that are based on the introduction and processing of test samples in flowing media.
A primary classification can be based on (a) the way the test portion is introduced, i.e. continuously or intermittently/discretely and (b) the basic character of the flowing media, i.e. either segmented, unsegmented or monosegmented, where segmentation is applied to prevent intermixing of successive analyte zones. This leads to the following classification tree (as in analytic compendium [12]):

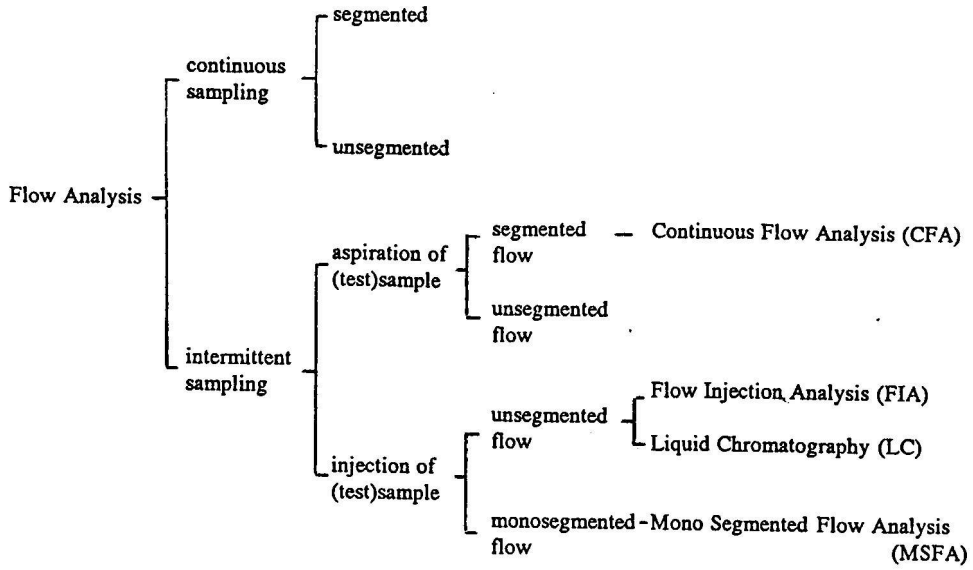


Fig. Classification scheme of flow methods of analysis.

50 fluorescence
Luminescence which occurs essentially only during the irradiation of a substance by electromagnetic radiation. [2]

51 fluorescence correlation assay
Assay in which fluctuations in fluorescence intensity are detected and used to characterize a species that is labeled with a fluorescent *probe*.
The fluctuations are measured for a small volume of the total assay sample and are attributed to local diffusion of the *probe* in the detected volume or interactions that change the quantum yield of fluorescence.

52 fluorescence polarization (*p*)
Unitless value determined from the fluorescence intensities (I) measured parallel ($I_{\parallel}$) and perpendicular ($I_{\perp}$) to the excitation plane. [1]

$$p = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + I_{\perp}}$$

Note: Fluorescence anisotropy (r) differs only in normalization and is determined from $r = (I_{\parallel} - I_{\perp}) / (I_{\parallel} + 2I_{\perp})$
Typically, fluorescence polarization is expressed as millipolarization ($mp = p \times 1000$).
Related term: *fluorescence polarization assay*

53 fluorescence polarization assay

Assay in which the association between an intrinsically fluorescent ligand, or a *ligand* linked to a fluorescent *probe*, and a larger macromolecule is detected. The degree to which the labeled *ligand* depolarizes plane-polarized light is directly proportional to the rate at which it tumbles in solution. Tumbling will be slower when the *ligand* is associated with the larger macromolecule as compared to free in solution, consequently resulting in a lower degree of depolarization.
Related term: *fluorescence polarization*, *homogeneous binding assay*.

54 fluorescence resonance energy transfer assay, (FRET assay)
Assay based on the non-radiative energy transfer that occurs when the emission spectrum of a fluorescent donor molecule overlaps with the absorption spectrum of an energy acceptor molecule. The donor and the acceptor fluorophores are linked directly or indirectly to two binding partners and energy transfer occurs when the fluorophores are in close proximity, i.e. when the binding partners interact.
Note 1: The fluorophores can be either of chemical or protein nature.
Note 2: Use of a donor with a long fluorescence lifetime allows time-delayed (or time-resolved) fluorescence measurement (TR-FRET), providing conditions where other fluorophores in the assay medium are not detected.

55 functional assay
Assay in which the biological or physiological activity of the target is measured.
Example: An assay in which an agonist stimulates, in receptor-transfected cells, the production of a second messenger which is detected with a fluorescence readout.

56 GTP-binding Protein Coupled Receptor (GPCR)
Family of transmembrane receptors that directly connects to a family of GTP binding protein/GTPases for signal transduction.

57 heterogeneous assay
Assay in which the response is detected only after the physical separation of one or more assay components.
Related terms: *heterogeneous binding assay*, *homogeneous assay*

58 heterogeneous binding assay
Assay in which one binding component is immobilized to a surface (e.g. cellular membrane, glass, gold or biopolymer layer) while its binding partner is freely diffusible. The extent of interaction between the binding partners is determined after their physical separation, which is usually achieved by filtration, centrifugation, magnetic field or chromatography.
Used to qualitatively discriminate specific and non-specific interaction or quantitatively determine the concentration of a component part of a mixture.

59 high content screening assay

Assay that produces multiple biological readouts.
Note: Most commonly used in relation to the mathematical (quantitative) analysis of an image acquired using an automated microscope. Analysis algorithms are used to quantify cellular parameters (e.g. number, motility, neurite outgrowth, size, shape) and subcellular events (e.g. receptor internalization, protein translocation, protein expression nuclei shape).

60 high performance liquid chromatography assay (HPLC assay)
Assay in which response is determined after the physical separation of assay components using high performance liquid chromatography (HPLC) with concurrent detection, usually by monitoring absorbance at an appropriate wavelength.
Changes in the concentration of assay components at one or more times over the course of the assay can be determined by the relative area under the detection peaks as compared to assay controls.

61 high throughput
Relative term, applied to the generation of a large number of results in a short timeframe (eg 100,000 in a week or a month). Usually achieved by employing some degree of *automation*.
Related terms: *throughput, ultra-high throughput.*

62 high throughput screening (HTS)
Method in which a large number of *assays* (from thousands to millions) is performed and assessed in a relatively short time period. Typically, these assays are carried out in microplates of at least 96 wells using automated or robotic technologies.
Note: The rate of at least 100,000 assays per day has been termed *ultra high throughput screening (UHTS).*

63 hit
Sample that produces confirmed activity above the hit threshold in an assay and whose structural identity has been confirmed.
A substance becomes a *hit* when the properties of an *active* are confirmed by elimination of *false positive* results and *artifacts.*
Note: In the past, the terms confirmed hit, true hit, and confirmed active were used with this meaning.

64 hit rate
Proportion of hits from a screen that displays confirmed activity beyond a minimum defined level, the *hit threshold*. It is expressed as a percentage of the number of samples screened.

65 hit threshold
Minimum activity that defines *actives* in a *primary screen*. It is usually expressed as percentage of inhibition or stimulation.

Example: a widely used hit threshold is 50% inhibition.

66 homogeneous assay
Assay in which all reagents and reactants are of a uniform phase and assay response is detected without the need for physical separation of assay components.
Related term: *heterogeneous assay*.

67 homogeneous binding assay
Single-phase system used to qualitatively discriminate between specific and non-specific interactions or quantitatively determine the concentration of a component part of a mixture.

68 IC_{50} (Inhibitory concentration 50)
The concentration of an enzyme inhibitor or receptor antagonist that reduces the enzyme activity or agonist response by 50%.
Note: IC_{50} values are influenced by experimental conditions (eg: substrate or agonist concentration – which should be specified).
Related terms: EC_{50}, *inhibition constant,* K_i.

69 image-based assay
Assay in which image analysis algorithms, that determine the compartmentalization of fluorescent probes, give topological as well as quantitative information.
Note: The use of automated cellular imaging for screening has been enabled by the development of digital imaging technology.
Related term: *high content screening*

70 immobilized metal ion affinity-based fluorescence polarization assay, (IMAP™ assay)
Assay in which the interaction between nanoparticles coated with trivalent metal ions and phosphate groups on a substrate that carries a fluorescent tag is detected. A phosphorylated fluorescent substrate bound to the nanoparticle will exhibit high fluorescence polarization relative to the unbound (unphosphorylated) peptide substrate.
Used for kinase assays where a phosphate group is added to a peptide substrate or for phosphatase assays where the phosphate is removed from a peptide substrate.
Related term: *fluorescence polarization*.

71 inactive n, adj
Sample that, at the tested concentration, does not produce a response above the hit threshold in an assay.
Note: A sample may also be designated as inactive when attempts to confirm an *active* fail.
Related terms: *artifact*, *false positive*, *hit*.

72 inhibition constant, K_i

1. Equilibrium dissociation constant of an enzyme-inhibitor complex: $K_i = [E][I]/[EI]$.
2. The equilibrium dissociation constant of a receptor-ligand complex.

Note: This value is usually obtained through competition binding experiments, where the K_i is determined after the IC_{50} obtained in a competition assay performed in the presence of a known concentration of labeled reference ligand (L_r) which has a known dissociation constant, $K_d[L_r]$, for the target: $K_i = IC_{50}/(1+([L_r]/K_d[L_r]))$. [13]

73 inhibitor

Compound that decreases the rate of a catalyzed reaction (enzyme) or transport process (transporter).

Characterized by an IC_{50} at the cellular or at the molecular level.

Note: The mechanism of inhibition can be assigned by experimentation. The two most commonly encountered mechanisms involve either competition with one of the substrates to occupy the active site (competitive inhibition), or binding at an alternative site inducing conformational changes in the active site (allosteric inhibition). Independently from the site of action, inhibitor binding can be reversible (the on and off rates are such that dissociation of the inhibitor occurs rapidly in relation to the timescales employed in the assay) or irreversible (most often covalent, as in the case of 'suicide substrates' which bind covalently to the active site upon activation by an enzyme).

74 inverse agonist

Ligand that decreases signaling through a receptor below the level of constitutive activity.

Note: Several receptors, especially when expressed at high levels, exhibit constitutively active signaling. Demonstrating an inverse agonist effect is dependent upon the constitutive activity of the particular *receptor* which is manifested in the absence of a conventional *agonist*. Upwards of 85% of *antagonists* identified in screens of GPCRs are actually inverse agonists. [14]

Related term: *agonist*, *partial agonist*, *antagonist*.

75 in vitro assay

Assay in which the component or components under study are removed from their complete biological systems.

Note: Literally "in glass".

Related term: *in vivo assay*

76 in vivo assay

Assay that involves a complete living organism.

Related term: *in vitro assay*

77 isotope

Atom of any particular element that has the same number of protons but differing numbers of neutrons in the nucleus.

Related terms: *radioisotope, isotopic labeling*, *radioligand*.

78 isotopic labeling
Incorporation of an *isotope* (radioactive or stable) into a molecule, by replacement of, or substitution at, one of its constituent atoms
See also *isotope*, *radioligand*, *probe*.

79 k_1
synonym: k_{on}
Rate of formation of the enzyme-substrate (ES) or ligand-receptor (LR) complex.
Expressed in units of $M^{-1}s^{-1}$.

80 k_{-1}
synonym: k_{off}
Rate of dissociation of the enzyme-substrate (ES) complex to enzyme (E) and substrate (S) or of ligand-receptor (LR) to receptor (R) and ligand (L).
Expressed in units of inverse time (s^{-1}).

81 kinetic assay
Assay in which the time-course of the signal intensity is measured. Signal values are typically acquired at several time intervals in order to calculate kinetic parameters.
Note: Kinetic assays are generally designed such that the change in signal is linear throughout the experiment.
Related terms: *equilibrium assay*, *end-point assay*.

82 label-free assay
Assay in which there is no requirement to modify one of the interacting components in order to facilitate signal detection.
Examples: Assays in which calorimetry, mass spectrometry or nuclear magnetic resonance provides the signal.
Note: Used to monitor macromolecule / macromolecule or small molecule / macromolecule binding, as well as cell adhesion or cell signaling.

83 label-free detection
Direct detection of compound activity on a target without the requirement for a labeled reagent.
Examples: Use of calorimetry, mass spectrometry or nuclear magnetic resonance to measure a signal.

84 laboratory information management systems (LIMS)
Computerized system designed to provide on-line information about the samples analyzed in a laboratory. Information provided may include the current location of each sample in the laboratory, the method and status of each analysis, and experimental data and calculated results.

Typically, LIMS connect analytical instruments to one or more personal computers and manage processes from sample log-in to reporting test results. With interfaces to enterprise resource planning or manufacturing execution systems a LIMS can be embedded in a complex IT-infrastructure. [15]

85 lead
Compound (or compound series) that satisfies predefined minimum criteria for further structure and activity optimization.
Note: Typically, a lead will demonstrate appropriate activity, selectivity, tractable SAR and the potential to be patentable.

86 lead identification
Process that is targeted toward the generation of at least one compound series that meets the requirements for progression to lead optimization. It typically encompasses the steps from the detection of initial activity (via high throughput screening and other lead finding activities) through hit confirmation and hit-to-lead activities.

87 lead optimization
Process in which the drug-like properties of an initial lead or lead series are improved. Typically, biological activity will be enhanced, in vivo efficacy will be demonstrated and compounds with a physicochemical, pharmacological and toxicological profile consistent with progression to the clinic will be identified.

88 library
1. Collection of samples (e.g. chemical compounds, natural products, over-expression library of a microbe) available for screening.
2. Set of compounds produced through combinatorial chemistry or other means which expands around a single core structure.

89 ligand
Any small molecule that binds to a larger molecule or macromolecular structure.
Related terms: *agonist*, *antagonist*, *channel blocker*, *channel opener*, *inhibitor*.

90 ligand activated channel
Any ion channel that is gated (i.e., opened or closed) by a *ligand*.
Note: Distinct from voltage-gated or stretch-activated ion channels.
Related term: *voltage activated channel*.

91 liquid handler
synonym: liquid handling machine
Automated device that accurately and precisely delivers programmed, pre-defined quantities of liquid to a *microplate*. It may be free-standing or incorporated as a *workstation* into a fully automated system.

Related term: *automated device*

92 luminescence assay

Assay in which response is measured by the detection of light of a non-radiative origin, such as bioluminescence or chemiluminescence.

Example: A luciferase reporter system for the measurement of gene transcription regulation in which luciferase, transcribed under the regulation of a promoter, oxidizes the substrate luciferin to oxyluciferin. In this process, light of wavelength 560nm is emitted and detected as the signal.

93 Michaelis constant, K_m

Concentration of substrate in an enzyme catalyzed reaction at which the rate of reaction is equal to one half of the limiting rate (maximum rate).

For the system:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES \xrightarrow{k_2} E + P$$

$$K_m = (k_{-1} + k_2)/k_1$$

94 microarray

Planar surface where assay reagents and samples are distributed as sub-microliter drops.

Note: This screening format is a direct offshoot of genomic microarray technologies and makes use of ultra-low volume *miniaturization* provided by nanodispensing technologies,

95 microplate

Any of a number of plates containing a series of wells which may be used for storage or to perform individual assays. Typically, these plates are constructed of a variety of clear and opaque plastics, and can contain from 6 to 3,456 individual wells.

Related terms: *microplate standards, assay, plate format*.

96 microplate standards

Defined footprint, height, flanges and well positions for 96, 384 and 1536 well *microplates*.

Note: These standards have been established by the Society for Biomolecular Sciences (SBS) and the American National Standards Institute (ANSI) and accredited by ANSI. They are numbered as ANSI-SBS-1 (2004) – Microplate footprint; ANSI-SBS-2(2004) – Microplate Height; ANSI-SBS-3 (2004) – Microplate Flange and ANSI-SBS-4 (2004) – Microplate Well Positions.

97 miniaturization

Experimental design aimed at decreasing the reaction volume of an assay and consequently the amount and cost of reagents.

Examples: Adaptation of assays from test tube or 96 well format to high density microplates (384 wells, 1536 wells), chip-based microarrays and microfluidic devices.

So-called nanodispensing systems may be required for liquid handling of submicroliter volumes.

98 module
Individual automated device within a fully automated assay system that usually performs a complete single assay step or procedure.
Note: A fully enclosed module may allow for the control of temperature, humidity and the gaseous environment.

99 molecular imprinted polymers (MIPS)
Synthetic polymers in which well-defined volume cavities (imprints) created by template molecules mimic a biomolecular specific ligand-receptor interaction. [15]
Note: These artificially generated recognition sites have shapes, sizes and functionalities complementary to the template molecule, and are capable of rebinding the template molecule in preference to other closely related structures.
Examples: MIPs have found uses as stationary phases in chromatography, as recognition elements in chemosensors, and as enzyme mimics in catalysis.

100 multiplex assay
Assay in which a single sample is used to measure the activity of more than one target in a single test.

101 n
1. Number of independent experiments.
2. Number of replicates, may or may not indicate experimental independence of the repeated observations.
Note: statistical treatment and interpretation of the data will depend on whether or not the experiments are independent. [17]

102 natural products
1. Complex mixtures derived from natural (biological) sources used in screening as a resource for identification of lead compounds.
2. Pure compounds of natural (biological) origin (whether obtained by purification of natural mixtures or by laboratory synthesis), as opposed to compounds originating from synthetic chemistry.
Typical origins include extracts from microbes and higher organisms from terrestrial or marine environments.

103 negative assay control
synonym: low control
Experimental conditions designed to produce the signal reflective of the absence of the signal under investigation.
Note: For a binding or an activation mechanism, it is typically the signal measured in the absence of a test compound and is often relevant to the determination of the *background*

signal. For an inhibitory or antagonist mechanism, it is the maximal signal, obtained in the absence of the reference inhibitor

104 noise
Random fluctuations occurring in a signal that are inherent in the combination of instrument and method. [2]
Related term: *background*

105 non-competitive binding assay
synonym: direct binding assay
Assay in which the interaction of a ligand with a receptor is measured without a requirement for added competing agents. It can be homogeneous or heterogeneous.

106 nuclease assay
Method in which fluorescently labeled 20 to 30 bp oligo probes are used in combination with the 5'→3' exonuclease activity of Taq polymerase to determine the presence or quantify the amount of specific target nucleotide sequences.
The 5' nuclease assay has now become a staple of real-time PCR quantification techniques. In the 5' nuclease assay, fluorescent signals are generated from the exponential range of the reaction, where component concentrations are not limiting. As a result, initial template levels can be determined with high accuracy.

107 orthosteric site
Binding site recognized by the endogenous agonist of a receptor.

108 pA2
Measure of antagonist potency. The negative logarithm (base 10) of the antagonist Molar concentration that shifts a submaximal point of the agonist concentration-response curve (usually the midpoint) to the right by a factor of two.

109 partial agonist
Agonist that is unable to induce a maximal receptor response in a given biological assay, regardless of the amount applied.
Related term: *efficacy*.

110 pipettor
Device that aspirates and dispenses liquid (n*L* – m*L* range) for the purposes of transferring from a source to destination.
Note: The device may be fully manual, electronic, single-channel, multi-channel (typically 8- or 12-channel), or part of a *liquid handler*.

111 plate format

Number and configuration of wells on a microplate. The most widely used formats are arrays of 96 wells (8 by 12) or 384 wells (16 by 24) or 1536 wells (32 by 48).
Related terms: *microplate*, *microplate standards*.

112 plate gripper
Handling device that positions *microplates* on a workstation. A plate gripper may also be used to move microplates between independent modules of an automated platform. Often used in combination with plate stackers, washers and readers.

113 plate map
Layout of samples and controls configured on a plate during an assay.
Example: For a primary screen in 384-well plates, columns 1, 2 and/or 23 and 24 are controls, and the remaining columns are for individual test compounds. For secondary screening more complex layouts are used and each row may contain a single compound at varying concentrations.

114 plate reader
Automated device that uses optical and/or computer vision techniques to detect biological, chemical or physical events in samples stored in microplates.
Note: Use of plate readers reduces or eliminates human subjectivity in the evaluation of plate contents.
Note: Often used in conjunction with a *plate stacker*.
Example: An absorbance plate reader measures color intensity in each well.

115 plate stacker
Automated device that loads unloads and restacks microplates. The device is usually part of system that integrates a plate reader, a liquid handler or other device to minimize microplate handling.

116 plate washer
Automated device used to wash the wells of microplates. The device usually has functions that allow precise height adjustment for minimized residual volume, and digitally controlled aspiration- or dispensing pumps to provide high accuracy.

117 positive assay control
synonym: high control
Experimental conditions designed to produce the signal reflective of maximum biological effect in an assay.
Note: For a binding or an activation mechanism, it is typically the signal measured in the presence of a test compound. For an inhibitory or antagonist mechanism, it is the minimum signal, obtained in the absence of the reference inhibitor.

118 potency

Qualitative expression for the activity of a drug, in terms of the concentration or amount needed to produce a defined effect as compared to a standard reference.
Note: More potent compounds have lower IC_{50} or EC_{50} values implying that less is needed for an effect.

119 precision
Closeness of agreement between independent test results obtained by applying the experimental procedure under stipulated conditions. The smaller the random part of the experimental errors which affect the results, the more precise the procedure. A measure of precision (or imprecision) is the standard deviation.
Related term: *accuracy*

120 primary screen
Initial screen applied to assess the activity of a collection of compounds and *identify hits* or *actives* against a biological target of interest.
This screen identifies *actives* from a *library*.
Related term: *secondary screen*.

121 probe
Molecule, chemical or protein which is covalently linked to the molecule, chemical or protein to be assayed and which is detected by an appropriate technology. A probe is often required since most assay techniques are indirect and require the use of a marker which is detected by the appropriate technology.
Most frequently used probes are radioactive or fluorescent, but alternative technologies are emerging (spin probes, heavy atoms).
Related terms: *isotopic labeling*

122 quality control
Operation or series of operations that contributes to the validation of screening results by establishing the acceptable limits of performance of internal controls.
Examples: Validation of liquid handling devices and plate readers, determination of the *Z' factor* and use of *assay controls*, and post-experiment controls.
Note: Results of a screen are validated only after a set of quality controls have been performed

123 radioisotope
Radioactive isotope of a specified element [2]
A radioisotope decays or disintegrates spontaneously, emitting radiation.
Related term: *isotope*.

124 radioligand
Ligand into which a *radioisotope* has been incorporated as a label.
Related terms: *isotope*, *isotopic labeling*.

125 receptor
Cellular macromolecule (or assembly of macromolecules) involved in chemical signaling within or between cells. A receptor recognizes a signal by binding a ligand with high affinity and chemical specificity, and then transduces a subsequent cellular response. The ligand may be exogenous (e.g., a drug) or endogenous (e.g., a hormone or neurotransmitter).

126 reproducibility
Closeness of agreement between independent results obtained with the same method on identical test material but in distinct experiments (different operators, different apparatus, different laboratories and/or after different intervals of time).
Note: A complete statement of reproducibility requires specification of the experimental conditions which differ. [2]

127 robot
Automated device that performs tasks (i.e. *screen* functions) that would normally be performed by a human. A robot in laboratory automation is usually used to move *microplates* in an automated assay procedure and usually consists of either a robotic arm with at least three degrees of freedom or a *plate gripper*.

128 robustness
Extent to which an assay or screen exhibits high discriminatory power and produces a low number of *false negative* and *false positive* results.

129 sample
Portion of material selected from a larger quantity of material. [2]
Example: a chemical compound or mixture of compounds submitted to an assay or a screen.

130 scheduling software
Computer program used to organize the chronological sequence of experimental methods to be followed to execute an automated high throughput screening campaign.
Based on the experimental needs of the campaign and constraints imposed by the programmer, this software uses an iterative process to determine the optimal order of the use of robots, liquid handlers, and modules to minimize screening time.

131 scintillation proximity assay (SPA™ assay)
Homogeneous assay in which beads incorporated with scintillant emit light in the presence of radiolabeled molecules within the proximity of the bead's scintillant detection.
Note: An alternative form of the assay incorporates the scintillant into the base of a microplate instead of a bead (FlashPlate™).

132 screen
Execution, analysis and interpretation of a large number of assays to evaluate the activity of a collection of samples against a target.
A screen will often employ automation.

133 screen validation
Assay conditions, as determined by *assay validation*, are performed in the chosen *plate format* with an acceptable signal to background ratio as described by the *Z' factor*.

134 secondary screen
Screen applied to independently confirm actives from the primary screen.
Note: A secondary screen may employ an assay that differs in type from the primary screen, e.g. biochemical assay vs. cell based assay, or it may be of the same type with different readout.

135 selectivity assay
Assay used to determine the relative potency of active or lead compounds towards an alternative target. A selectivity assay (or panel of assays) may include targets of the same family or unrelated targets.

136 standard error mean (SEM)
Standard deviation divided by the square root of the sample size. It is the standard deviation of a sample of means.

137 stably transfected cells
Eukaryotic cells into which recombinant DNA has been introduced and incorporated into the genome, so that the cells replicate the new DNA in a stable fashion.
Note: The biological material thus obtained is expected to show low variability and, therefore, to increases assay reproducibility.

138 structure-activity relationship (SAR)
Association between specific aspects of molecular structure and defined biological action. [2]

139 sublibrary
Portion of a *library* that is grouped or selected based on similarity of structure or biological effect.

140 surface plasmon resonance assay (SPR assay)
Assay in which the binding of a ligand to a protein target immobilized on the gold surface of a chip is detected by changes in the angle of the light reflected from the surface.

Note: This assay type is not usually used for *high throughput screening* but has been expanded recently to four simultaneous detection channels and has improved sensitivity to detect low molecular weight binding molecules.

141 target
Biological molecule, such as an enzyme or receptor, whose activity and function is the focus of a screen.

142 targeted library
Library designed, on the basis of preexisting information, to generate enhanced activity or hit rate against a particular biological target or target class.

143 throughput
Number of results that can be generated in a given timeframe. In *high throughput screening (HTS)*, throughput is often defined by the number of assay samples that can be processed in a day (e.g. 50,000 samples per day).
Related terms: *high throughput, ultra-high throughput.*

144 total internal reflection fluorescence (TIRF)
Spectroscopic technique that discriminates between labeled molecules in the homogeneous phase and those interacting with binding partners immobilized at a surface. TIRF measurements are restricted to a thin layer and are achieved by exciting the fluorophore with an evanescent wave, created by total internal reflection at the glass/ aqueous interface of the wave guide. The goal of using TIRF in biological applications is to study events close to the interface of two different media.

145 toxic effect
Deleterious effect on cell or animal viability in a functional assay.

146 transiently transfected cells
Eukaryotic cells into which recombinant DNA has been introduced and expressed by cellular transcription, but without incorporation into the stable genome.

147 ultra-high throughput
Relative term, currently applied to the screening of 100,000 test samples in a 24 hour period.
Related terms: *throughput, high throughput.*

148 V_{max}
Maximum velocity that an enzyme catalyzed reaction can achieve for a particular substrate under a given set of conditions.

Note: $V_{max} = k_2[E]_0$ where k_2 is the rate of product formation and $[E]_0$ is the total enzyme concentration. This equation is derived from a mathematical analysis of the kinetic scheme:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES \xrightarrow{k_2} E + P$$

by applying specific assumptions regarding the conditions of the experiment, e.g. $k_{-1} >> k_2$, [S] >> [E]

149 voltage activated channel

Ion channel that is specifically activated, or gated, by the surrounding potential difference near the channel (or near the cell, neuron or synapse).

150 workstation

Programmable device used to automate a single operation (such as liquid handling) on a *microplate* supplied to the device by a *robot* (automated stacker system or a robotic manipulator).[18]

151 *Z* factor

Dimensionless statistical parameter that provides a practical assessment of assay performance in the presence of test compounds.

$$Z = 1 - ((3\sigma_s + 3\sigma_c) / (| \mu_s - \mu_c |)$$

Where μ_s denotes the mean of the library sample signal, μ_c denotes the mean of the control signal and σ_s and σ_c denote the corresponding standard deviations.
$1 > Z \geq 0.5$ generally categorizes an excellent assay. [19]

152 *Z′* factor

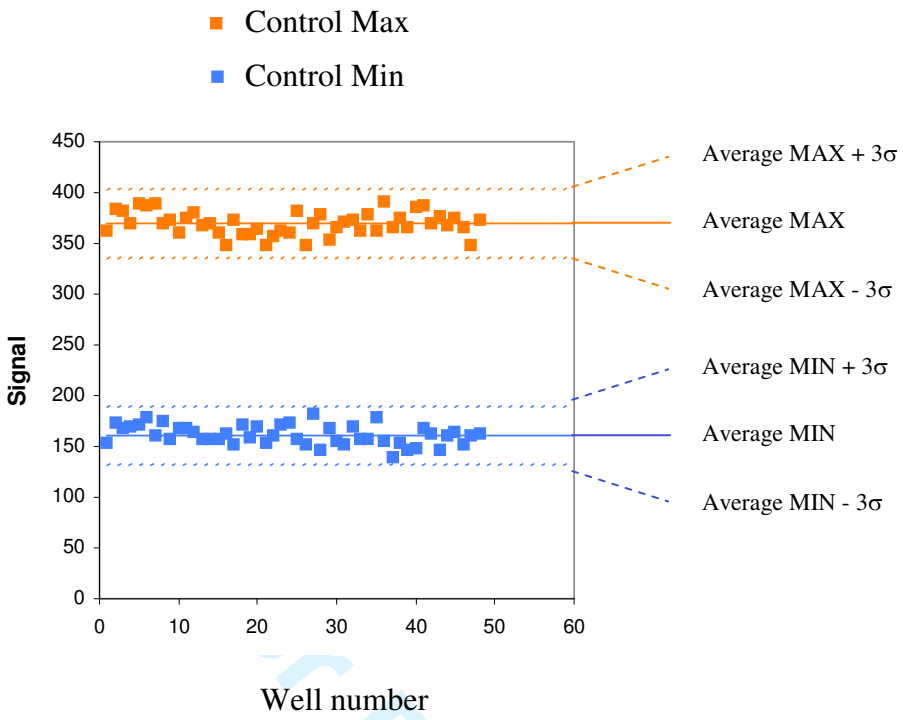
Dimensionless statistical parameter that is used extensively in biomolecular screening. The Z' factor is a characteristic of an assay without the intervention of test compounds. It provides a practical index of the quality and reliability of the assay, and can be used as a guide to assay development and optimization.

$$Z' = 1 - (3\sigma_{c+} + 3\sigma_{c-}) / (| \mu_{c+} - \mu_{c-} |)$$

where σ_{c+} and σ_{c-} are the standard deviations of the high and low controls and μ_{c+} and μ_{c-} are the means of the high and low controls, respectively.

The useful range of Z' values is from 0 (very poor) to +1 (excellent). In most cases, a Z' factor greater than 0.5 is required for an assay to be accepted for HTS.[19]

A graphical illustration of the parameters used in the calculation of Z'is provided below.



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