U03E-H10E

Dyes and Stains, Indicators or Probes or Labels for cell biology and biochemistry

Dyes, stains, labels, and indicators terms are used with more or less accurate meaning in the laboratory, in different applications. Let's do some categorization:

Stains are substances used, beside large industry applications to stain materials (cosmetics, paper,…), to color distinguishly substances in complex samples in biosciences . For example, hematoxylin is used to visualize nucleic acids in tissues sections, while eosin visualizes other cell components. Ethidium bromide is used similarly in cells, but more commonly to track visually DNA or RNA an agarose gel after electrophoresis. Stains are used in various microbiological assays as well as in histological applications.

Colored and fluorescent **Indicators** are dyes used for their property to change their color (absorbency or fluorescence) in presence of particular parameters or substances. For example phenol red is a red dye useful to visualize the acidification of cell culture media or setting pH of solutions (titration), shifting to yellow. 3,5-DIBR-PAESA #474660 allows for colorimetrically dosing Fe2+ and Co3+.

Cell biology colored or fluorescent **Probes** include indicators and stains which affinity allow to detect specifically (to visualize and even measure) particular physiological parameters or cell or tissues structures, such as the intracellular pH, the membrane lipidic bilayer of organelles, the cytoskeleton, cytoplasmic Ca2+, caspases enzymes,… Typically a probe changes its property by interaction, as an indicator -or not, as a marker or label-. Probes more or less specific for bacteria, living or dead cells, pathological tissues such as in MultipleSclerosis or Amyloidosis, are rather called stains.

Colored and fluorescent **Labels** are used extensively in fluorescent techniques to tag biomolecules and visualize them, but also eventually to handle them (purification). Fluorescein is the most original and pioneer fluorescent label, but is also used as an indicator because its fluorescence can be affected by pH (in particular once derivatize adequately, i.e. with AcetoxyMethyl ester), or by oxygen reactive species (as in the H2DCFDA reagent). Furthermore, bridging labels to probes make themn change in fluorescence (color, intensity) depending on the environment, or after chemical reactions. This includes enzyme- mediated reactions (see fluorescent Enzyme Substrates & Reagents).

Finally, the term **Dye** is used similarly as stain (in industry) or as label (in biotechs). Dyes are colored or fluorescent by themselves, and used to dye materials, by their own affinity properties (like a stain) or by chemical coupling (like a label). A dye bears a color by itself (chromophore or fluorophore). To make a difference, a stain indicates its dying ability (and can be non colored when alone); a label indicates its ability to be distinguishable (by a colour, a fluorescence, an enzyme, a tag,…); a chromogenic or fluorigenic or luminogenic compound can generate a colour or a fluorescence or light.

More information and examples:

- **Dyes and Stains**.
- **pH Indicators** (and their color/pH Transition i.e. for culture media)
- **Labels**
- **Cell Probes & Indicators** for **pH**, for **ions** (Ca2+, Mg2+, Na/Cl/K, Metals (Zn2+, Fe2+, Co2+, …),
  for **cell structures** (membranes, organelles,…)
  for **cell metabolism and state** (respiration, viability, apoptosis, trafficking, signaling,…)

Application examples by types of fluorescent compounds:

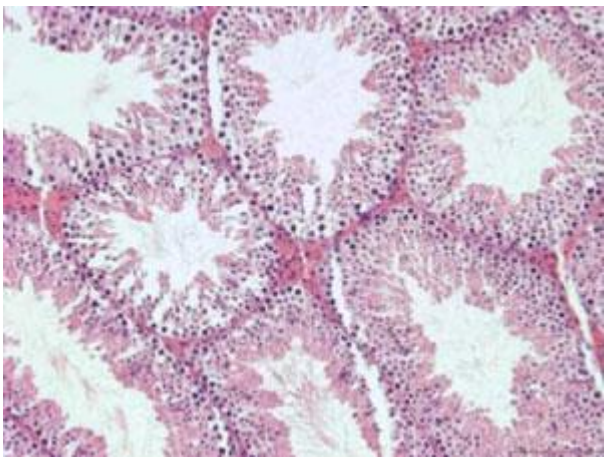
One can distinguish compounds that fluorescence in a stable way (labels) from those which fluorescence depends on the environment or buffer characteristics (pH indicators). Other interfere with macromolecules (stains) or more specifically with cell structures (cell probes) while other interfere with smaller molecules such as Ca2+, Cl- or Zn+ (probes, indicators).

Dyes and Stains

Dye or stain generally colored of fluorescent compounds used to visualize in different analytical techniques (microscopy, electrophoresis,…) more or less specifically certain types of components in samples: proteins or DNA/RNAs in cells, bacteria or tissues, gels,... (more specific stains are more often called probes)
Widely used dyes in molecular biology include for examples **Coomassie® brilliant blue** (000000) that stains proteins in SDS PAGE or **ethidium bromide** (89244B) for staining DNA/RNA.
Coomassie® brilliant blue binds to practically all proteins, and is therefore useful to visualize protein bands separated on a polyacrylamide gel. In addition, this substance can be used to detect lipids in TLC, or to track water uptake in the roots of plants. Ethidium bromide binds to double stranded nucleic acid so that these molecules become detectable under UV light.

Histological investigations rely on a variety of stains. The most common staining technique used here is probably the **Hematoxylin-Eosin** staining (H&E). This allows the nuclei to be stained with a dark bluish or purple color due to its interaction with the dye Hematoxylin (091922), while the cytoplasmic components to stain pink due to Eosin interaction. This stain binds preferably to the nucleus because it is more basophilic. The rest of the cell tends to be more acidophilic leading it to interact with the more acid substance, eosin.

Neutral red (09341U) is another dye used in histology for staining nuclei red. Neutral red is also used in cell viability tests based on the ability of viable cells to incorporate and bind neutral red.



H&E stain of murine testes section.

Dyes are also used in microbiology to make microorganisms visible or differentiate them due to distinct staining properties. **Crystal violet** (N1275A) and **safranine** (N1282A) are used in Gram's stain, a staining technique used to classify bacteria in which a bacterial specimen is first stained with crystal violet, then treated with an iodine solution, decolorized with alcohol, and counterstained with safranine. Gram-positive bacteria retain the violet stain, gram-negative bacteria do not.

Search stains online for Histology/Immuno-Chemistry/Fluorescence[]

Indicators

Indicators are used to measure typically ions concentration in cells (I.e. Ca2+ flux/signals; heavy metals ions), or in solutions (and notably the pH).

● Ion indicators

As typical example of a pH indicator, **bromocresol green** (3,3',5,5'-tetrabromo-m-cresolsulfonphthalein) (039820) is frequently used as an indicator in acid-base titrations. The actual indicator is the sodium salt of the sulfonic acid. Since sulfonic acids are strong acids, the basicity of the SO3- group can be ignored. The absorption maxima for each of the anions formed can be measured at 455nm (yellow) and at 630nm (blue). The pH range that can be measured due to the acidity of the solution is from 3.8 to 5.4.
When bromocresol green is used as an indicator, a second acidic dissociation occurs resulting in the formation of a dianion. This dianion is actually a resonance hybrid comprised of several different structures.
At their isosbestic point of 52nm both species have the same molar absorptivity, and the total absorbance of a solution containing the two ions is independent of their relative concentrations but dependent on the total dye concentration. The measured absorbance is the sum of the two contributing ions.
In addition bromocresol green can be used as a tracking dye in agarose gels. In alkaline agarose gels it is easier to see than the more commonly used bromophenol blue dye indicator. Finally, thanks to binding properties, it has also been used to detect proteins, in particular albumin.

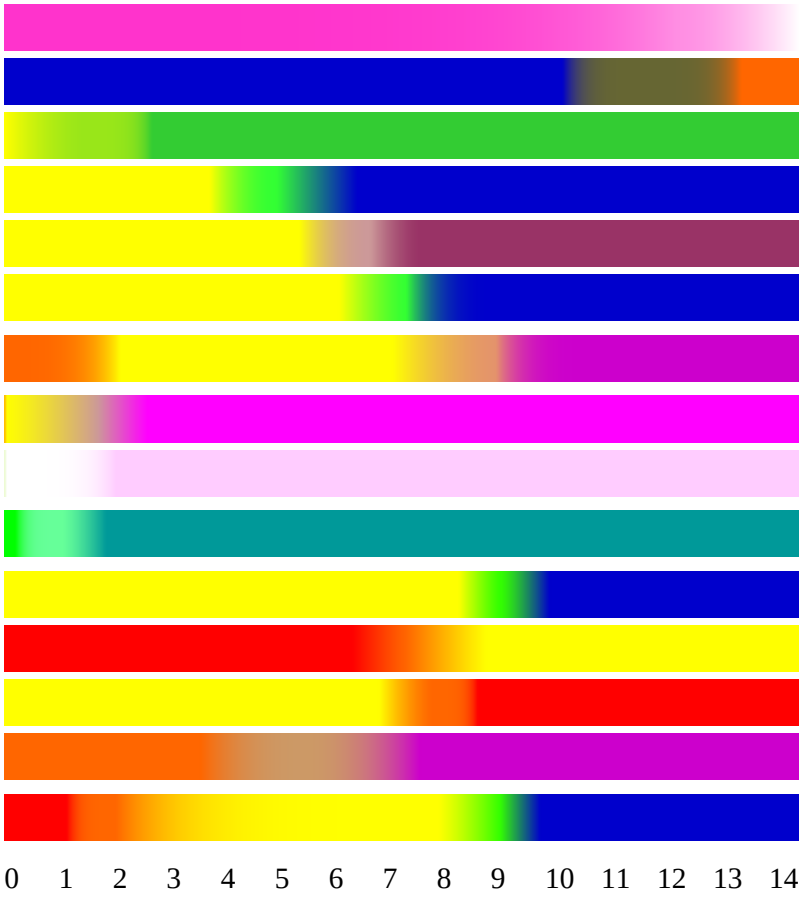
Indicators are added to microbial culture media to detect metabolic properties of microorganisms. **Neutral red** (09341U), in addition to its use as nuclear stain, is also a pH-indicator, the color of its acid form is red, the color of the base form is yellow. MacConkey agar for example, contains neutral red and lactose. Colonies formed by bacteria that ferment lactose into lactic acid will turn red and stand out from the pale colonies of non-lactose-fermenting bacteria.

Indicators used also in culture media, including **bromocresol green** and **phenol red** (JQ9130) and many more.

List of indicators.
See Cell Biology Staining and Probing [PH]. See also page Cell probes for pH and ions[]

●Color-Transition pH Indicators
Common pH indicators for the culture media with their color transitions:

Cat.#	Indicator	Transition	pH range with transition
A-2000	Acid fuchsin	12.0 - 14.0	
A-7910	Aniline blue	10.0 - 13.0	
B-5500	Brilliant green	0.3 - 2.6	
B-7900	Bromocresol green	3.8 - 5.4	
B-7920	Bromocresol purple	5.2 - 6.8	
B-8987	Bromothymol blue	6.0 - 7.6	
C-8565	Cresol red	1.8 - 2.0/7.0 - 8.8	
C-8650	Crystal violet	0.0 - 2.0	
E-2100	Eosin Y	0.0 - 1.7	
M-0300	Malachite green Oxalate	0.2 - 1.8	
M-3598	Methylene blue	8.0 - 9.6	
N-2525	Neutral red	6.8 - 8.0	
P-2800	Phenol red	6.8 - 8.2	
R-4000	Resazurin	3.8 - 6.5	
T-3846	Thymol blue	1.2 - 2.8/8.0 - 9.2	



Aniline blue #61572x is used to stain collagen fibers in tissue sections using the Masson′s trichrome protocol. It can be used in the Mallory's connective tissue stain and Gömöri trichrome stain. It is used in differential staining, where Collagen is stained blue. It has also been used to stain and visualize glycosylated proteins., and to reveal callose structures in plant tissues.
Many indicators are alos used as probes or stains. I.e. Aniline Blue-Azan (Heidenhain's) Stain Kit #FTT620

Labels

Fluorescent labels or other tags are useful tools in the tracking substances within cells, or isolating a desired substance based on the presence of a specific tag which can be detected directly under a microscope using the appropriate filters. Probably the most common label in use today is **Fluorescein** , as its isothiocyanate Isomer I (FITC) (19365A) or other derivatives. FITC once conjugated to an antibody or other probes, can be detected in the green range in several applications ranging from immunohistochemistry to cell sorting. The use of varying fluorescent tags allows the experimenter to analyze different structures, proteins, or cells in a single experiment. Other classic fluorochromes include Rhodamines and Coumarins, and notably the CYanine dyes, while modern fluorescent labels include the superior FluoProbes dyes, CF dyes, DL dyes and AF dyes.
Fluorescent labels visible at different wavelengths have been very useful in the area of cell trafficking research, for example.
Other fluorochome compounds strongly fluoresce only when bound, for example **Dansyl** chloride (01395A) that react with the terminal amino group of a protein or peptide. This labeling agent is used to make Fret-pair detections. In biochemistry, after acid hydrolysis of all the other peptide bonds, the terminal amino acid is identifiable as the dansylated residue.

More: see page fluorescent labeling.

Probes for Cell Structure and Physiology

A biology Probe has useful affinity for a specific physiological parameter or biology structures, such as the intracellular pH and ions, cytoplasmic Ca2+, organelles or membrane lipids, the cytoskeleton, caspases enzymes,…
This affine property is beared by molecular configuration (shape and/or ionic/hydrophobic motif), a chelate moiety, a peptide, an oligonucleotide, an antibody,…
These probes allow to detect, visualize, track in real-time and even measure cell parameter of significant interest, because they bear their own color or fluorescence or other means (spin-labels), or can be detected by indirect methods (tags), or eventually without any label (SPR).

Many dyes and stains described above can be used as ±specific probes for some samples and applications. Main chromogenic and fluorigenic probes are presented in the Cell Biology Staining and Probing catalog[PH].

See also web pages of Cell Biology []:
Probes for pH and ions[](Ca2+, Mg2+, Na/Cl/K, Metals (Zn2+, Fe2+, Co2+, …),
Cell Structures probes[](cytoskeleton, organelles,…),
Cell Viability[], Apoptosis[], Oxidative Metabolism[](ROS, NOs, GST,…), Cell Signaling[](ions, GPCR, Hormones,…), …

Rem: some probes are sometimes called **markers** or even **biomarkers**, but these terms should be rather reserved to the detected molecules -affinity-targets- related to an application (ex: PS is an apoptosis marker detected by (labeled)Annexin probes; AFP and CEA are cancer biomarkers).

Related products/documents

Products HighLights Overview, including:
Remarkable conjugation tools: PEO crosslinkers (hydrophilic spacers), Hydrazone chemistry (flexible method using stable activation step), Click Chemistry & Staudinger ligation (versatile and mild conjugation), Multifunctional cross-linkers

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