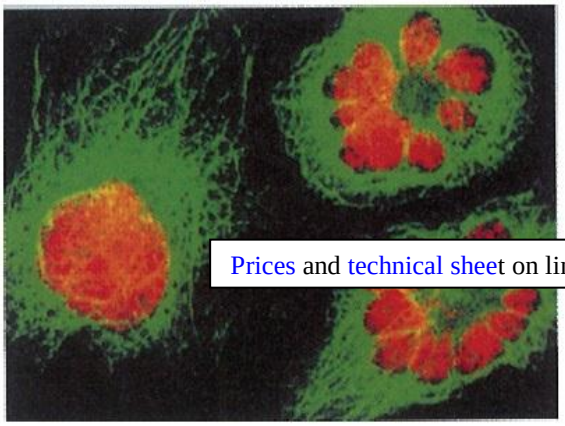
H10E

CellStain solutions

- **Ready-to-use high quality** staining solutions for cells in microscopy techniques



Prices and technical sheet on line

-Cellstain- DAPI solution **BE8260, 1ml**
4',6-Diamidino-2-phenylindole, dihydrochloride, aqueous solution; CAS: 28718-90-3; MW: 350.25, C16H17Cl2N5 (L)
2.9 mM DAPI buffer solution (1 mg DAPI/1 ml) (slightly yellow to yellow liquid)

-Cellstain- AO solution **RI6430-, 1ml**
3,6-Bis(dimethylamino)acridine, hydrochloride, aqueous solution; CAS; MW: 301.81
Orange or yellow solution 3.3 mM AO (1 mg AO/1 ml water)

-Cellstain- EB solution **T31440, 1ml**
(M)

-Cellstain- PI solution **367740, 1ml**
3,8-Diamino-5-[3-(diethylmethylammonio)propyl]-6-phenylphenanthridinium diiodide, aqueous solution, CAS: 25535-16-4(PI), MW: 668.39
Orange to red solution 1.5 mM PI (1 mg/1ml water (M)

-Cellstain- Calcein-AM solution **855425, 1ml**
3',6'-Di(O-acetyl)-4',5'-bis[N,N-bis(carboxymethyl)aminomethyl]fluorescein tetraacetoxymethyl ester, DMSO solution CAS: 148504-34-1 (Calcein-AM); MW: 994.86, C48H46N2O23
Colorless liquid 1 mM Calcein-AM in DMSO (1.0 mg/1ml DMSO) (J)

-Cellstain- CytoRed solution **T30820, 1ml**
7-Isobutyloxycarbonyloxy-3H-phenoxazin-3-one; MW: 313.31, C17H15NO5 (J)
1 mM CytoRed DMSO solution (yellowish-orange)

-Cellstain- Hoechst33258 solution **BD6061, 1ml**
Bisbenzimide, 2'-(4-Hydroxyphenyl)-5-(4-methyl-1-piperazinyl)-2,5'-bi-1H-benzimidazole, trihydrochloride, solution; CAS: 23491-45-4; MW: 533.88, C25H27Cl3N6O (L)
1 mg/ml aqueous solution (yellow solution)

-Cellstain- Hoechst33342 solution **BE8270, 1ml**
Bisbenzimide, 2'-(4-Ethoxyphenyl)-5-(4-methyl-1-piperazinyl)-2,5'-bi-1H-benzimidazole, trihydrochloride, solution; CAS: 23491-52-3 (free base); MW: 561.93, C27H31Cl3N6O (L)
1 mg/ml aqueous solution (Yellow liquid)

-Cellstain- MitoRed solution **T32840, 50µgx8vials**
9-[2-(4'-Methylcoumarin-7'-oxycarbonyl)phenyl]-3,6-bis(diethylamino)xanthylium chloride;MW: 637.17, C38H37ClN2O5 (L)
Red purple to purplish-brown solid

-Cellstain-Double Staining Kit (Calcein & PI) / Live & Dead cells **486301, Kit**
To simultaneously stain and observe live and dead cells by microscopy. λex.535 nm, λem.617 nm. Contains 200 and 300µL of rgt A and B to stain ~250 slides Technical sheet

See also Vital stains

Detailed information

Cellstain Live/Dead staining kit

-Cellstain-Double Staining Kit (Calcein & PI) / Live & Dead cells **486301, Kit**

To simultaneously stain and observe live and dead cells by microscopy. λex.535 nm, λem.617 nm. Contains 200 and 300µL of rqt A and B to stain ~250 slides Technical sheet

-Cellstain-Double Staining Kit is utilized for simultaneous fluorescence staining of viable and dead cells. This kit contains Calcein-AM and Propidium Iodide (PI) solutions, which stain viable and dead cells, respectively (Fig. 1). Calcein-AM, an acetoxymethyl ester of calcein, is highly lipophilic and cell membrane permeable. Although Calcein-AM itself is not a fluorescent molecule, the calcein generated from Calcein- AM by esterase in a viable cell emits a strong green fluorescence (excitation: 490 nm, emission: 515 nm). Therefore, Calcein-AM only stains viable cells. On the other hand, PI, a nuclei staining dye, cannot pass through a viable cell membrane. It reaches the nucleus by passing through disordered areas of dead cell membrane and intercalates with the DNA double helix of the cell to emit red fluorescence (excitation: 535 nm, emission: 617 nm). Since both calcein and PI-DNA can be excited with 490 nm, simultaneous monitoring of viable and dead cells is possible with a fluorescence microscope. With 545 nm excitation, only dead cells can be observed (Fig. 2). Since optimal staining conditions differ from cell line to cell line, we recommend that a suitable concentration of PI and Calcein- AM be individually determined. Please note that PI is suspected to be highly carcinogenic; careful handling is required.

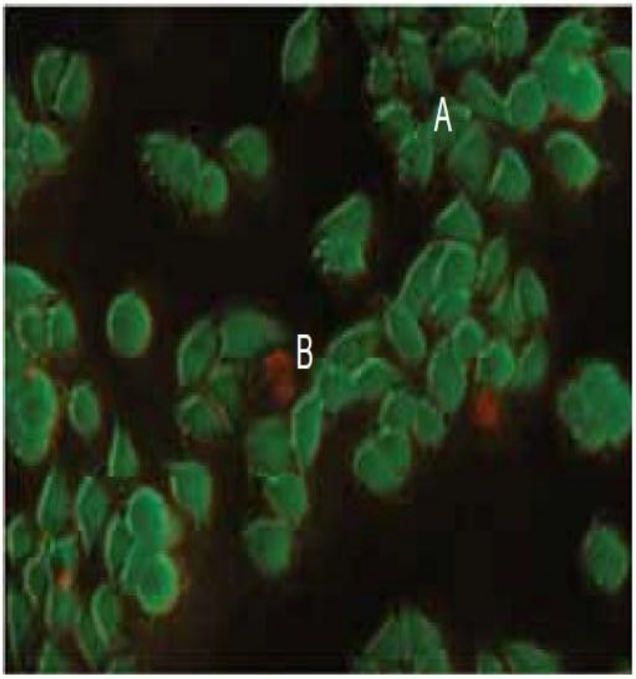


Fig. 2 Cell staining with Double Staining Kit
HeLa cell, incubated with assay solution for 15 minutes.
A) viable cell B) dead cell

Required Equipment and Materials:
Microscope with 490 nm excitation filter and 530 nm emission filter,
Glass slide or glass bottom plate, CO2 incubator, 10 µl and 200 µl adjustable pipettes, PBS

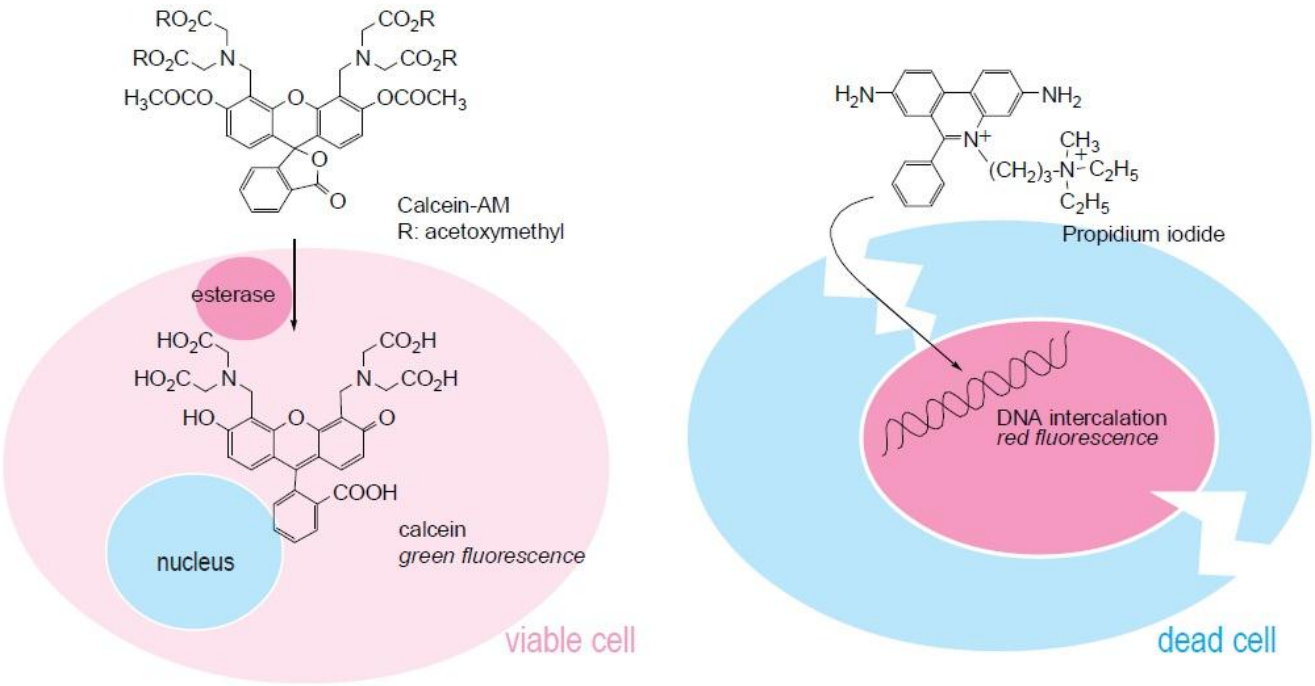


Fig. 1 Assay system to determine viable cells and dead cells

AO Cellstain

-Cellstain- AO solution **RI6430-, 1ml**

3,6-Bis(dimethylamino)acridine, hydrochloride, aqueous solution; CAS: 65-61-2; MW: 301.81
Orange or yellow solution 3.3 mM AO (1 mg AO/1 ml water)

Acridine orange (AO) forms a complex with double-stranded DNA to emit green fluorescence (Fig. 1). AO also forms a complex with singlestranded DNA or RNA to emit red fluorescence. One molecule of AO intercalates with three base pairs of double-stranded DNA and emits green fluorescence with the maximum wavelength at 526 nm (excitation 502 nm). One molecule of AO can also interact with one phosphate group of single-stranded DNA or RNA to form an aggregated, or stacked, structure that emits red fluorescence with the maximum wavelength at 650 nm (excitation 460 nm). Therefore, AO is utilized for the detection of both double-stranded DNA and single-stranded DNA or RNA. It enables simultaneous determination of DNA and RNA with argon laser excitation or flow cytometry.

powder: A386-10, 1mg
reddish-brown powder (L)

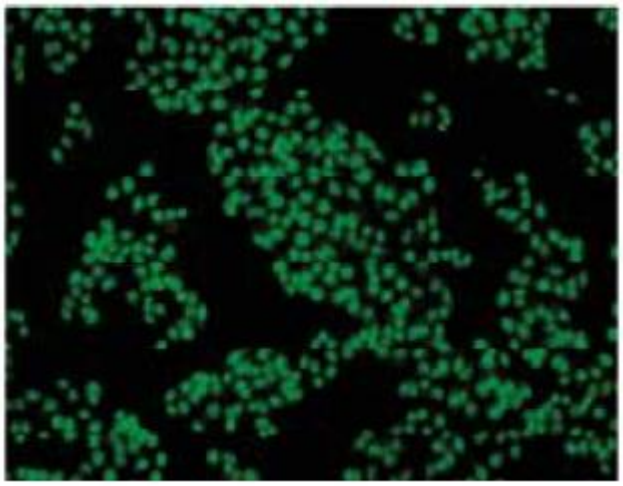


Fig. Cell staining with AO
Cell type: HeLa

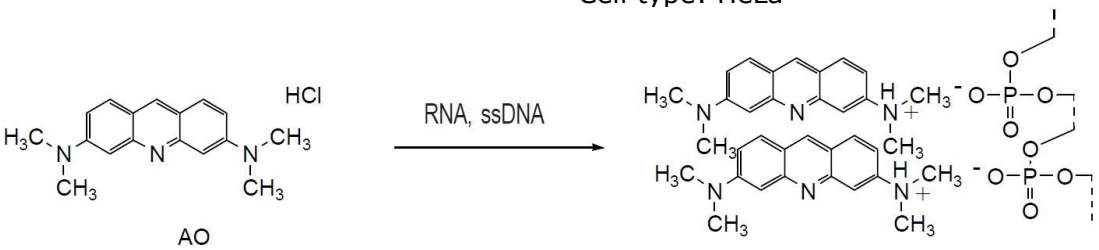


Fig. 1 Cell staining mechanism

Staining Procedure

1. Prepare 10-50 µM AO solution with PBS or an appropriate buffer.[a)]
2. Add AO solution with 1/10 of the volume of cell culture medium to the cell culture.[b)]
3. Incubate the cell at 37ºC for 10-20 min.
4. Wash cells twice with PBS or an appropriate buffer.
5. Observe the cells under a fluorescence microscope with 500 nm excitation and 530 nm emission filters.

a) Since AO may be carcinogenic, extreme care is necessary during handling.
b) You may replace the culture medium with 1/10 concentration of AO buffer solution.

References

1. I. W. Taylor, et al., An Evaluation of DNA Fluochromes, Staining Techniques, and Analysis for Flow Cytometry. I. Unperturebed Cellpopulations. J Histochem Cytochem. 1980;**28**:1224-1232.
2. N. Miyoshi, et al., Fluorescence Lifetime of Acridine Orange in Sodiu Dodecyl Sulfate Premicellar Solutions. Photochem Photobiol. 1988;**47**:685-688.
3. A. K. El-Naggar, et al., Single- and Double-stranded RNA Measurements by Flow Cytometry in Solid Neoplasms. Cytometry. 1991;**12**:330-335.
4. Y. Miyakoshi, et al., The Frequencies of Micronuclei Induced by Cisplatin in Newborn Rat Astrocytes Are Increased by 50-Hz, 7.5- and 10-mT Electromagnetic Fields. Environ Health and Prev Med. 2005;**10**:138-143.

BCECF Cellstain

-Cellstain- BCECF - powder: 45440Z-B262

3'-O-Acetyl-2',7'-bis(carboxyethyl)-4 or 5-carboxyfluorescein, diacetoxymethyl ester CAS:117464-70-7; MW: 688.59, C35H28O15 (M)
orange or orange-brown crystals, >90%(HPLC)

BCECF is the most widely used intracellular pH probe. Dr. Tsien and others improved this carboxyfluorescein by introducing two extra carboxylates that allow it to be retained better by the cell. BCECF is highly water-soluble because it has 4 to 5 negative charges at neutral pH; it becomes difficult to pass through the cell membrane after loading. Its pKa value, 6.97, is higher than that of carboxyfluorescein. BCECF has an isosbestic point at 439 nm in the excitation spectra, so it can be used in ratiometry, similar to Fura 2. Wavelengths of 505 nm and 439 nm are usually used for the ratiometric assay, and 490 nm and 450 nm filters are set in front of the excitation light source. The 530 nm filter is used for its fluorescent signal. Please note that the excitation spectrum is slightly different from the absorption spectra. BCECF-AM is an acetoxymethyl ester of BCECF that enables easy loading of BCECF into cells. BCECF-AM accumulates in a cell only by incubation as do the other acetoxymethyl esters. BCECF-AM is very sensitive to moisture; it should be carefully handled. The color of the DMSO solution changes from pale yellow to dark orange with decomposition of the AM form. Therefore, hydrolysis of the AM ester can be monitored by changes in color.

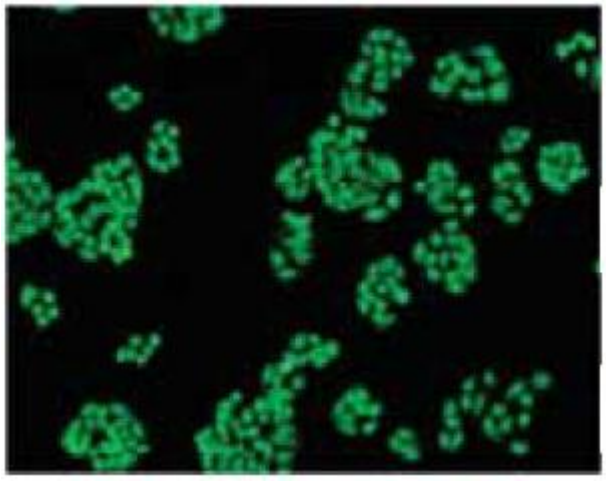
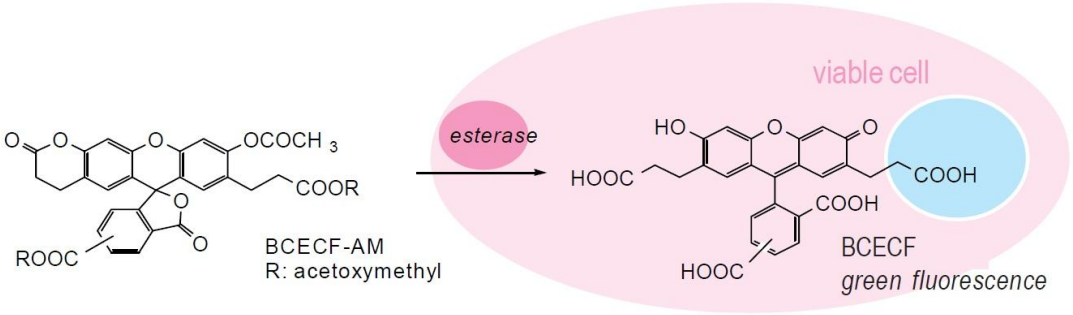


Fig. Cell staining with BCECF (Heal type cells)

BCECF cell staining mecanism:



General Protocol (for Human Neutrophil)*

Reagents: 1 mM BCECF-AM/DMSO solution (1 mg BCECF in 1.45 ml DMSO)
HEPES buffer saline (20 mM HEPES, 153 mM NaCl, 5 mM KCl, 5 mM glucose, pH 7.4)

Protocol:

1. Suspend cells in HEPES buffer solution to prepare $4x10^7$ cells per ml.
2. Add 1 mM BCECF-AM/DMSO solution to the cell suspension to prepare 3 µM BCECF-AM (1/300 vol of cell suspension) as the final concentration.
3. Incubate the cell suspension at 37ºC for 30 minutes.
4. Wash the cells 3 times with HEPES buffer saline and then prepare $3x10^6$ cells per ml of the cell suspension.
5. Determine the fluorescence intensity using a fluorescence microscope or a confocal laser microscope coupled with an image analyzer.

* Cell staining conditions depend on cell type, so it is necessary to optimize the conditions for each experiment

References

1. R. A. Steinhardt, et al., Development of K^+-conductance and Membrane Potentials in Unfertilized Sea Urchin Eggs After Exposure to NH_4OH. Nature. 1973;**241**:400-401.
2. T. J. Rink, et al., Cytoplasmic pH and Free Mg^{2+} in Lymphocytes. J Cell Biol. 1982;**95**:189-196.
3. A. M. Paradiso, et al., Na^+-H^+ Exchange in Gastric Glands as Measured with a Cytoplasmic-trapped, Fluorescent pH Indicator. PNAS. 1984;**81**:7436-7440.
4. S. Grinstein, et al., Phorbol Ester-induces Changes of Cytoplasmic pH in Neutrophils: Role of Exocytosis in Na^+ - H^+ Exchange. Am J Physiol. 1985;**248**:C379-C386.
5. G. B. Zavoico, et al., Regulation of intracellular pH in human platelets. Effects of thrombin, A23187, and ionomycin and evidence for activation of Na^+/H^+ exchange and its inhibition by amiloride analogs. J Biol Chem. 1986;**261**:13160-13167.
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7. C. Aalkjaer, et al., Intracellular pH Regulation in Resting and Contracting Segments of Rat Mesenteric Resistance Vessels. J Physiol. 1988;**402**:391-410.
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- Exchange for Regulation of Intracellular pH in Renal Epithelial Cells. Biochim Biophys Acta.
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12. K. Khodakhah, et al., Functional Heterogeneity of Calcium Release by Inositol Triphosphate in Single Purkinje Neurones, Cultured Cerebellar Astorocytes, and Peripheral Tissues. PNAS. 1993;**90**:4976-4980.

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13. G. Boyarsky, et al., Superiority of in vitro Over in vivo Calibrations of BCECF in Vascular Smooth Muscle Cells. FASEB J. 1996;**10**:1205-1212.
14. S. A. Weston, et al., New Fluorescent Dyes for Lymphocyte Migration Studies Analysis by Flow Cytometry and Fluorescent Microscopy. J Immunol Methods. 1990;**133**:87-97.
15. L. S. De Clerck, et al., Use of Fluorescent Dyes in the Determination of Adherence of Human Leucocytes to Endothelial Cells and the Effects of Fluorochromes on Cellular Function. J Immunol Methods. 1994;**172**:115-124.

Calcein Cellstain

-Cellstain- Calcein-AM solution **855425, 1ml**

3',6'-Di(O-acetyl)-4',5'-bis[N,N-bis(carboxymethyl)aminomethyl]fluorescein tetraacetoxymethyl ester, DMSO solution CAS: 148504-34-1 (Calcein-AM); MW: 994.86, C48H46N2O23 (J)
Colorless liquid 1 mM Calcein-AM in DMSO (1.0 mg/1ml DMSO) powder: A326 white or slightly white powder (M)

Calcein-AM readily passes through the cell membrane of viable cells because of its enhanced hydrophobicity compared to Calcein. After Calcein-AM permeates into the cytoplasm, it is hydrolyzed by esterases to Calcein, which remains inside the cell (Fig. 1). Among other reagents, including BCECF-AM and Carboxy-fluorescein diacetate, Calcein-AM is the most suitable fluorescent probe for staining viable cells because of its low cytotoxicity. Calcein does not inhibit any cellular functions such as proliferation or chemotaxis of lymphocyte. In addition, viability assays using Calcein are reliable and correlate well with the standard 51Cr-release assay. The excitation and emission wavelengths of calcein are 490 nm and 515 nm, respectively

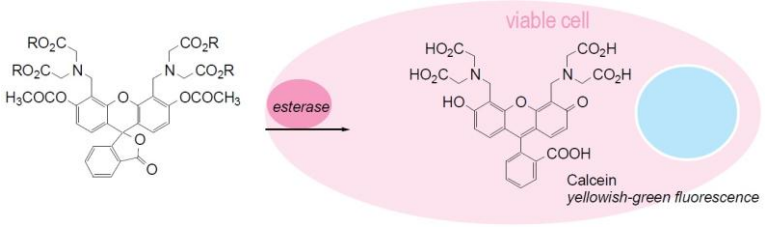

Fig. Cell staining mecanism

Staining Procedure

1. Prepare 1 mM Calcein-AM solution with DMSO and dilute to prepare 1-50 µM Calcein-AM solution with PBS.[a)]
2. Add Calcein-AM solution with 1/10 of the volume of cell culture medium to the cell culture.[b)]
3. Incubate the cell at 37ºC for 15-30 min.
4. Wash cells twice with PBS or an appropriate buffer.
5. Observe the cells under a fluorescence microscope with 490 nm excitation and 515 nm emission filters.

[a)] If the Calcein-AM has difficulty loading into cells, use a detergent such as Pluronic F127.
[b)] Or you may replace the culture medium with 1/10 concentration of Calcein-AM buffer solution.

Reference

1. K. McGinnes, et al., A Fluorescence NK Assay Using Flow Cytometry. J Immunol Methods. 1986;**86**:7-15.
2. S. J. Morris, Real-time Multi-wavelength Fluorescence Imaging of Living Cells. BioTechniques. 1990;**8**:296-308.
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5. H. Xie, et al., Intercellular Communication Through Gap Junctions Is Reduced in Senescent Cell. Biophys J. 1992;**62**:45-47.
6. S. A. Weston, et al., Calcein: a Novel Marker for Lymphocytes Which Enter Lymph Nodes. Cytometry. 1992;**13**:739-749.
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CFSE Cellstain

-Cellstain- CFSE solution **-, ml**

5- or 6-(N-Succinimidyloxycarbonyl)-fluorescein 3',6' diacetate; CAS: 150347-59-4; MW: 557.46, C29H19NO11 (M)

powder: A375,1mg white of slighlty white solid (M)

CFSE is cell-membrane permeable and readily accumulates inside viable cells where it covalently attaches to intracellular proteins (Fig. 1). Hydrolyzed CFSE emits fluorescence and covalently attached fluorescein molecules do not leak from cells. CFSE-labeled cells can be monitored over several weeks in vivo. Therefore, CFSE is utilized for detection of viable cell as well as for the long-term observation of cell activities by fluorescent microscopy. The excitation and emission wavelengths of CFSE-labeled cells are 500 nm and 520 nm, respectively. CFSE-stained cells are shown in Fig. 2.

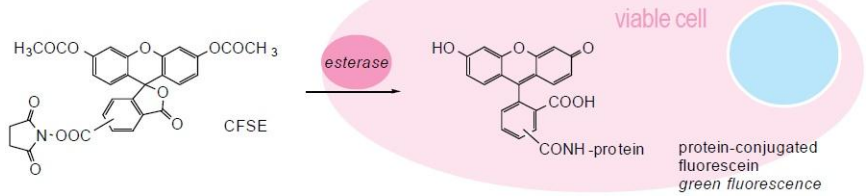

Fig. Cell staining mecanism

Staining Procedure

1. Prepare 1 mM CFSE solution with DMSO. Dilute it to prepare 10-50 μM CFSE solution with PBS or an appropriate buffer.
2. Add CFSE solution with 1/10 of the volume of cell culture medium to the cell culture.
3. Incubate the cell at 37ºC for 15 to 30 min.
4. Wash cells twice with PBS or an appropriate buffer.
5. Observe the cells under a fluorescence microscope with 490 nm excitation and 530 nm emission filters.

Reference

1. M. Bronner-Fraser, Alterations in Neural Crest Migration by a Monoclonal Antibody That Affects Cell Adhesion. J Cell Biol. 1985;**101**:610-617.
2. A. Nose, et al., A Novel Cadherin Cell Adhesion Molecule:Its Expression Patterns Associated WithImplantation and Organogenesis of Mouse Embryos. J Cell Biol. 1986;**103**:2649-2658.
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5. G. Radcliff, et al., Quantification of Effector/Target Conjugation Involving Natural Killer(NK) or Lymphokine Activated Killer(LAK) Cells by Two-color Flow Cytometry. J Immunol Methods. 1991;**139**:281-292.
6. S. A. Weston, et al., Calcein: a Novel Marker for Lymphocytes Which Enter Lymph Nodes. Cytometry. 1992;**13**:739-749.
7. L. S. D. Clerck, et al., Use of Fluorescent Dyes in the Determination of Adherence of Human Leucocytes to Endothelial Cells and the Effects of Fluorochromes on Cellular Function. J Immunol Methods. 1994;**172**:115-124.

CytoRed Cellstain

-Cellstain- CytoRed solution **T30820, 1ml**

7-Isobutyloxycarbonyloxy-3H-phenoxazin-3-one; MW: 313.31, C17H15NO5 (J)
1 mM CytoRed DMSO solution (yellowish-orange)

CytoRed is cell membrane permeable and accumulates inside of viable cells as resorufin. CytoRed has a much wider spectrum than BCECF or Calcein, so filters for fluorescein and rhodamine can also be used. The excitation and emission wavelengths of resorufin are 560 nm and 590 nm, respectively.

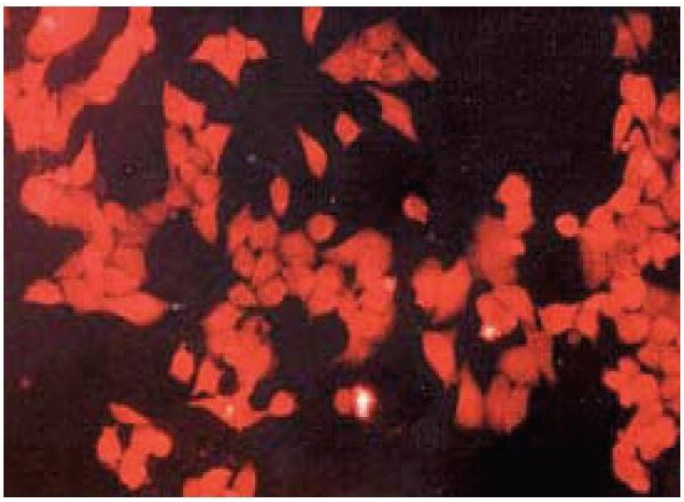
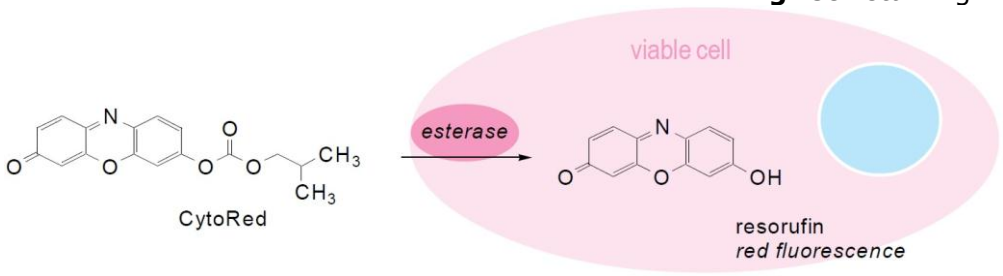


Fig. Cell staining with CytoRed (Hela Cell Type)

CytoRed Cell Stain mecanism:



Staining Procedure

1. Prepare 1 mM CytoRed solution with DMSO. Dilute it to prepare 10 μM CytoRed solution with culture medium or an appropriate buffer.[a)]
2. Prepare a $1x10^5$-$1x10^6$ cells/ml cell suspension and culture the cells in a chamber slide.
3. Remove culture medium and wash cells with culture medium (PBS-Hanks medium, etc).
4. Add CytoRed solution to the cells, and incubate the chamber at 37ºC for 30 min to 1 hour.
5. Remove the culture medium from cells and add new medium.[b)]
6. Wash cells twice with PBS or an appropriate buffer.
7. Observe the cells under a fluorescence microscope with 560 nm excitation and 590 nm emission filters.

a) Incubate the MitoRed buffer solution at 37ºC prior to adding to cells.
b) For fixing after washing cells, add 10% formarin buffer and incubate for 15-20 min, and then wash with PBS.

Reference

1. M. Ishiyama, et al., A Resorufin Derivative as a Fluorogenic Indicator for Cell Viability. Anal Sci. 1999;**15**:1025-1028.

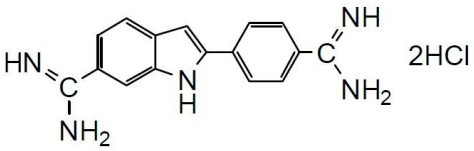
DAPI Cellstain

-Cellstain- DAPI solution **BE8260, 1ml**

4',6-Diamidino-2-phenylindole, dihydrochloride, aqueous solution; CAS: 28718-90-3; MW: 350.25, C16H17Cl2N5 (L)
2.9 mM DAPI buffer solution (1 mg DAPI/1 ml) (slightly yellow to yellow liquid)

Powder:.D523-10 Yellow powder or solid

DAPI is an AT-sequence specific DNA intercalator that attaches to DNA at the minor groove of the double helix like Hoechst dyes. Though DAPI is not permeable through viable cell membranes, it passes through disturbed cell membranes to stain the nucleus. DAPI has a high photo-bleaching tolerance level. DAPI is utilized for the detection of mitochondrial DNA in yeast, chloroplast DNA, virus DNA, micoplasm DNA and chromosomal DNA. The excitation and emission wavelengths of DAPI-DNA complex are 360 nm and 460 nm, respectively.



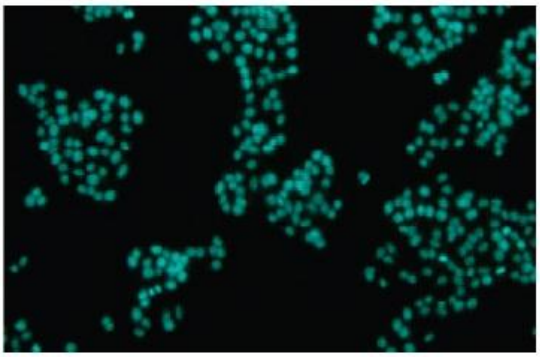


Fig. Cell staining with DAPI

Staining Procedure

1. Prepare 10-50 μM DAPI solution with PBS or an appropriate buffer.[a)]
2. Add DAPI solution with 1/10 of the volume of cell culture medium to the cell culture.[b)]
3. Incubate the cell at 37°C for 10-20 min.
4. Wash cells twice with PBS or an appropriate buffer.
5. Observe the cells using a fluorescence microscope with 360 nm excitation and 460 nm emission filters.

a) Since DAPI may be carcinogenic, extreme care is necessary during handling.
b) Or you may replace the culture medium with 1/10 concentration of DAPI buffer solution.

Reference

1. W. Schnedl, et al., DIPI and DAPI: Fluorescence Banding with Only Negligible Fading. Hum Genet. 1977;**36**:167-172.
2. I. W. Taylor, et al., An Evaluation of DNA Fluochromes, Staining Techniques, and Analysis for Flow Cytometry. I. Unperturebed Cellpopulations. J Histochem Cytochem. 1980;**28**:1224-1232.
3. F. Otto, et al., A Comparative Study of DAPI, DIPI, and Hoechst 33258 and 33342 as Chromosomal DNA Stains. Stain Technol. 1985;**60**:7-11.
4. N. Poulin, et al., Quantitative Precision of an Automated Image Cytometric System for the Measurement of DNA Content and Distribution in Cells Labeled with Fluorescent Nucleic Acid Stains. Cytometry. 1994;**16**:227-235.
5. M. Kawai, et al., Rapid Enumeration of Physiologically Active Bacteria in Purified Water Used in the Pharmaceutical Manufacturing Process. J Appl Microbiol. 1999;**86**:496-504.

FDA Cellstain

-Cellstain- FDA **-**

Fluorescein diacetate; CAS: 596-09-8; MW: 416.38, C24H16O7 (M)

FDA is cell-membrane permeable and accumulates inside of viable cells as fluorescein (Fig. 1). Since fluorescein is less hydrophilic than BCECF or Calcein, the leakage of fluorescein from cells is rather high. FDA is also utilized for flow cytometry. The excitation and emission wavelengths of fluorescein are 488 nm and 530 nm, respectively.

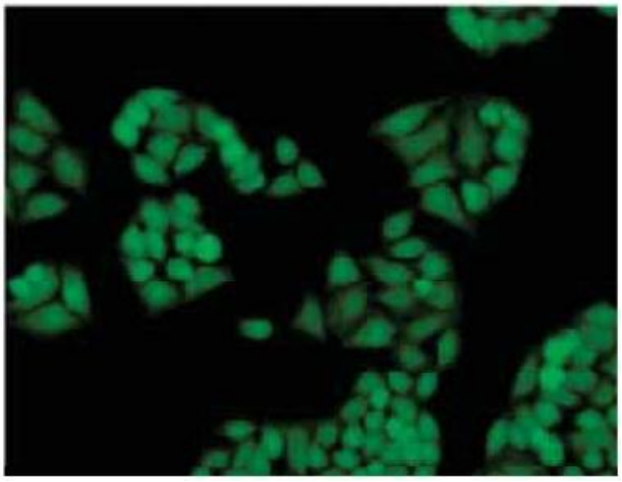


Fig. Cell staining with FDA

Staining Procedure

1. Prepare 0.5 mg/ml FDA stock solution with DMSO. Dilute 10 ul of the stock solution with 5 ml PBS(-).
2. Prepare a cell suspension and wash cells with PBS(-). Prepare $1x10^5$-$1x10^6$ cells/ml cell suspension
3. Add 15 ul FDA solution to 30 ul cell suspension, and incubate at 37ºC for 15-30 min.
4. Put 10 ul stained cell suspension on a glass slide and cover with a cover glass.
5. Observe the cells under a fluorescence microscope with 488 nm excitation and 530 nm emission filters.

Reference

1. B. Rotman, et al., Membrane Properties of Living Mammalian Cells as Studied by Enzymatic Hydrolysis of Fluorogenic Esters. PNAS. 1966;**55**:134-141.
2. H. R. Hulett, et al., Cell Sorting: Automated Separation of Mammalian Cells as a Function of Intercellular Fluorescence. Science. 1969;**166**:747-749.
3. K. H. Jones, et al., An Improved Method to Determine Cell Viability by Simultaneous Staining with Fluorescein Diacetate-Propidium Iodide. J Histochem Cytochem. 1985;**33**:77-79.
4. K. McGinnes, et al., A Fluorescence NK Assay Using Flow Cytometry. J Immunol Methods. 1986;**86**:7-15.
5. W. M. J. Vuist, et al., Potentiation by Interleukin 2 of Burkitt's Lymphoma Therapy with Anti-Pan B (Anti-CD19) Monoclonal Antibodies in a Mouse Xenotransplantation Model. Cancer Res. 1989;**49**:3783-3788.
6. E. Prosperi, Intracellular Turnover of Fluorescein Diacetate. Influence of Membrane Ionic Gradients on Fluorescein Efflux. Histochem J. 1990;**22**:227-233.

Cellstain

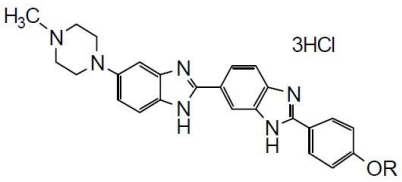
-Cellstain- Hoechst33258 solution **BD6061, 1ml**

Bisbenzimide, 2'-(4-Hydroxyphenyl)-5-(4-methyl-1-piperazinyl)-2,5'-bi-1H-benzimidazole, trihydrochloride, solution; CAS: 23491-45-4; MW: 533.88, $C_{25}H_{27}Cl_3N_6O$ (L)
1 mg/ml aqueous solution (yellow solution)

-Cellstain- Hoechst33342 solution **BE8270, 1ml**

Bisbenzimide, 2'-(4-Ethoxyphenyl)-5-(4-methyl-1-piperazinyl)-2,5'-bi-1H-benzimidazole, trihydrochloride, solution; CAS: 23491-52-3 (free base); MW: 561.93, $C_{27}H_{31}Cl_3N_6O$ (L)
1 mg/ml aqueous solution (Yellow liquid)

Hoechst dyes are cell membrane permeable and stain DNA to emit intense blue fluorescence. They bind to DNA in the minor groove of poly-AT sequence rich areas. Both Hoechst 33342 and Hoechst 33258 are water-soluble and stable in aqueous solutions. The excitation and emission wavelengths of Hoechst-DNA complex are 350 nm and 460 nm, respectively.



Hoechst 33258: R=OH
Hoechst 33342: R=C_2H_5

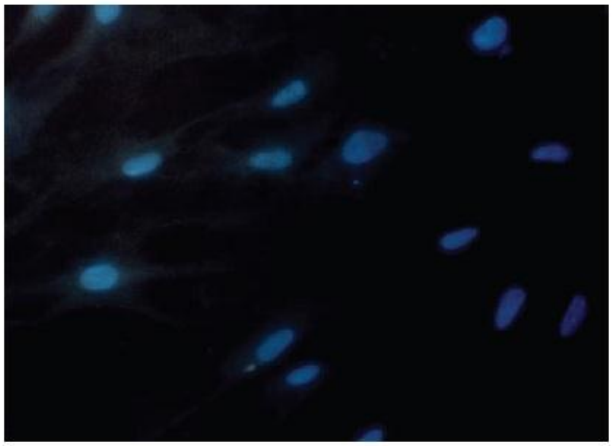


Fig. Cell staining with Hoechst 33258
Cell type: human fetal cell

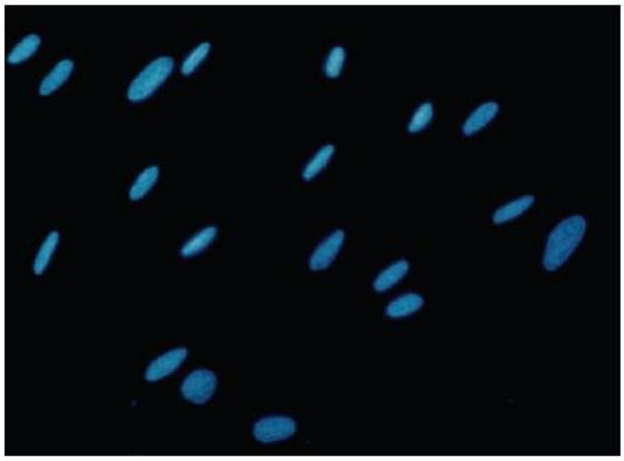


Fig. Cell staining with Hoechst 33342
Cell type: human fetal cell

Staining Procedure

1. Prepare 10-50 µM Hoechst dye solution with PBS or an appropriate buffer.[a)]
2. Add Hoechst dye solution with 1/10 of the volume of cell culture medium to the cell culture.[b)]
3. Incubate the cell at 37ºC for 10-20 min.
4. Wash cells twice with PBS or an appropriate buffer.
5. Observe the cells under a fluorescence microscope with 350 nm excitation and 460 nm emission filters.

a) Since Hoechst dyes may be carcinogenic, extreme care is necessary during handling.
b) Or you may replace the culture medium with 1/10 concentration of Hoechst dye buffer solution.

Reference

1. M. J. Lydon, et al., Vital DNA Staining and Cell Sorting by Flow Microfluorometry. J Cell Physiol. 1980;**102**:175-181.
2. M. Sriram, et al., Structural Consequences of a Carcinogenic Alkylation Lesion on DNA: Effect of O6-ethylguanine on the Molecular Structure of the d(CGC[e6G]AATTCGCG)-netropsin Complex. Biochemistry. 1992;**31**:11823-11834.
3. Y. Tadokoro, et al., Characterization of Histone H2A.X Expression in Testis and Specific Labeling of Germ Cells at the Commitment Stage of Meiosis with Histone H2A.X Promoter-Enhanced Green Fluorescent Protein Transgene. Biol Reprod. 2003;**69**:1325-1329.
4. F. Wada, et al., Analyses of Expression and Localization of Two Mammalian-Type Transglutaminases in Physarum polycephalum, an Acellular Slime Mold. J Biochem. 2004;**136**:665-672.
5. T. Ohara, et al., FoSTUA, Encoding a Basic Helix-Loop-Helix Protein, Differentially Regulates Development of Three Kinds of Asexual Spores, Macroconidia, Microconidia, and Chlamydospores, in the Fungal Plant Pathogen Fusarium oxysporum. Eukaryot Cell. 2004;**3**:1412-1422.

MitoRed Cellstain

-Cellstain- MitoRed solution **T32840, 50µgx8vials**

9-[2-(4'-Methylcoumarin-7'-oxycarbonyl)phenyl]-3,6-bis(diethylamino)xanthylium chloride; Rhodamine B 4-methylumbelliferyl ester chloride; MW: 637.17, C38H37ClN2O5 (L)
Red purple to purplish-brown solid; λex: 569 nm, λem: 594 nm in DMSO

MitoRed is a cell-membrane-permeable, rhodamine-based dye. It localizes in mitochondria and emits red fluorescence. The interaction of MitoRed with mitochondria depends on the membrane potential of the mitochondria. Mitochondria can be stained with 20 to 200 nM MitoRed. The excitation and emission wavelengths of MitoRed are 560 nm and 580 nm, respectively.

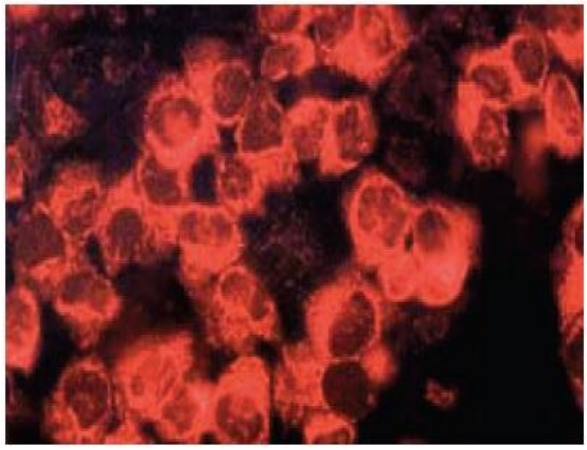
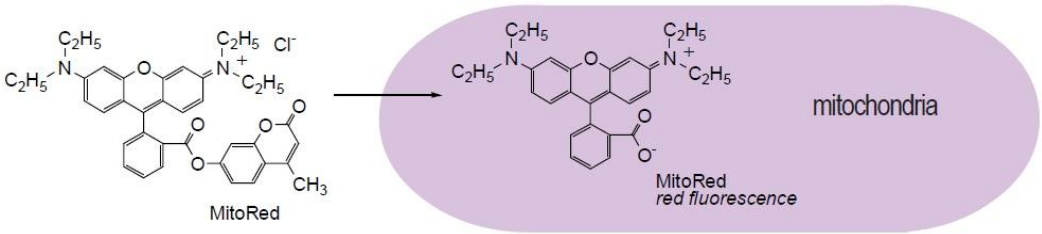


Fig. Cell staining with MitoRed

Cell staining mecanism of MitoRed:



Staining Procedure

1. Dissolve 50 µg MitoRed (1 tube) in 78 µl of DMSO to prepare 1 mM MitoRed-DMSO solution.
2. Prepare cells with a glass slide. The cell number should be $5x10^4$ to $5x10^5$ cells per ml.
3. Incubate the slide and wash cells with PBS or Hank's medium.
4. Dilute the 1 mM MitoRed solution with culture medium to prepare 20-200 nM MitoRed buffer solution.
5. Add the MitoRed buffer solution[a)] to the glass slide and incubate at 37ºC for 30 min to 1 hour.
6. Remove the MitoRed buffer solution and wash cells with culture medium.[b)]
7. Observe the cells under a fluorescence microscope with a rhodamine filter.

a) Incubate the MitoRed buffer solution at 37°C prior to adding to cells.
b) For fixing after washing cells, add 10% formarin buffer and incubate for 15-20 min, and then wash with PBS.

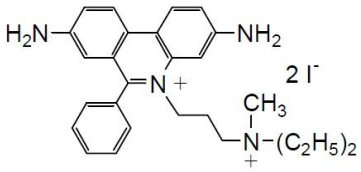
PI Cellstain

-Cellstain- PI solution **367740, 1ml**

3,8-Diamino-5-[3-(diethylmethylammonio)propyl]-6-phenylphenanthridinium diiodide, aqueous solution, CAS: 25535-16-4(PI), MW: 668.39 powder: A386-10, 1mg

Orange to red solution 1.5 mM PI (1 mg/1ml water (M) reddish-brown powder or solid (L)

Propidium iodide (PI) is an ethidium bromide analog that emits red fluorescence upon intercalation with double-stranded DNA. PI does not permeate viable cell membranes, but passes through disturbed cell membranes and stains the nuclei. PI is often used in combination with a fluorescein compound, such as Calcein-AM or FDA, for simultaneous staining of viable and dead cells. The excitation and emission wavelengths of PI-DNA complex are 535 nm and 615 nm, respectively.



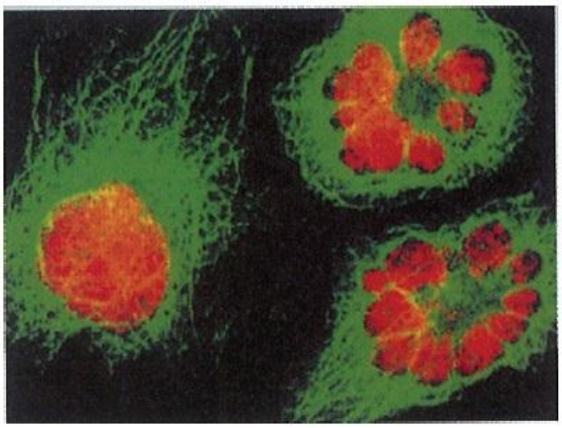


Fig. Cell staining with PI

Staining Procedure

1. Prepare 10-50 µM PI solution with PBS or an appropriate buffer.[a)]
2. Add PI solution with 1/10 of the volume of cell culture medium to the cell culture.[b)]
3. Incubate the cell at 37°C for 10-20 min.
4. Wash cells twice with PBS or an appropriate buffer.
5. Observe the cells under a fluorescence microscope with 535 nm excitation and 615 nm emission filters.

a) Since PI may be carcinogenic, extreme care is necessary during handling.
b) Or you may replace the culture medium with 1/10 concentration of PI buffer solution.

Reference

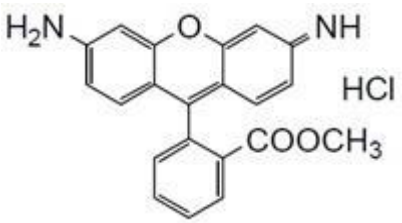
1. I. W. Taylor, et al., An Evaluation of DNA Fluochromes, Staining Techniques, and Analysis for Flow Cytometry. I. Unperturebed Cellpopulations. J Histochem Cytochem. 1980;**28**:1224-1232.
2. W. M. J. Vuist, et al., Potentiation by Interleukin 2 of Burkitt's Lymphoma Therapy with Anti-Pan B (Anti-CD19) Monoclonal Antibodies in a Mouse Xenotransplantation Model. Cancer Res. 1989;**49**:3783-3788.
3. A. K. El-Naggar, et al., Single- and Double-stranded RNA Measurements by Flow Cytometry in Solid Neoplasms. Cytometry. 1991;**12**:330-335.
4. C. Souchier, et al., Methods for Cell Proliferation Analysis by Fluorescent Image Cytometry. Cytometry. 1995;**20**:203-209.
5. T. Irino, et al., Establishment of Real-Time Polymerase Chain Reaction Method for Quantitative Analysis of Asparagine Synthetase Expression. J Mol Diagn. 2004;**6**:217-224.

Rh123 Cellstain

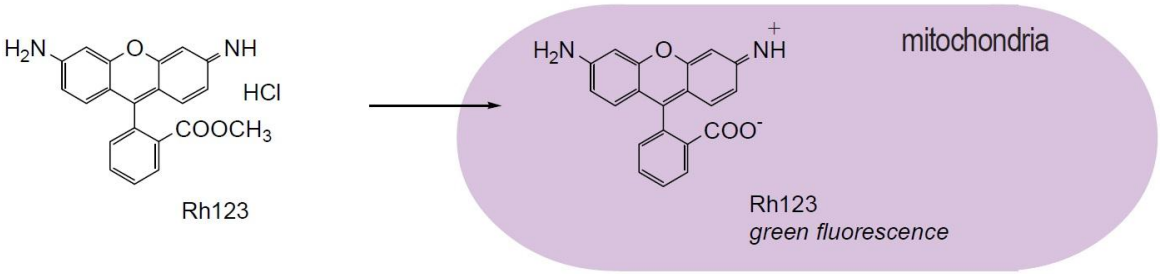
-Cellstain- Rh123 solution -

Rhodamine 123, 2-(6-Amino-3-imino-3H-xanthen-9-yl)benzoic acid methyl ester, hydrochloride [cas# : 62669-70-9]; CAS: 62669-70-9; MW: 380.82, C21H17ClN2O3 (L)
Red to reddish-brown powder or solid

Rhodamine 123 (Rh123) is cell-membrane permeable and localizes in mitochondria of viable cells to emit yellowish-green fluorescence (Fig. 1). Rh123 is utilized for staining a wide variety of cells, including plant cells and bacteria. Since there is a correlation between the amount of ATP in a cell and the fluorescence intensity of Rh123, this compound is used for the detection of intracellular ATP. Rh123 is also used in cancer research.



Cell Stain mecanism of RH123:



Staining Procedure

1. Dissolve 0.4 mg Rh123 in 1 ml DMSO to prepare 1 mM Rh123-DMSO solution.
2. Prepare cells with a glass slide. The cell number will be $5x10^4$ to $5x10^5$ cells per ml.
3. Incubate the slide and wash cells with PBS or Hank's medium.
4. Dilute the 1 mM Rh123 solution with culture medium to prepare 1-20 µM Rh123 buffer solution.
5. Add the Rh123 buffer solution[a)] to the glass slide and incubate at 37°C for 30 min to 1 hour.
6. Remove the Rh123 buffer solution and wash cells with culture medium.[b)]
7. Observe the cells under a fluorescence microscope with a fluorescein filter.

a) Incubate the Rh123 buffer solution at 37°C prior to adding to cells.
b) For fixing after washing cells, add 10% formarin buffer and incubate for 15-20 min, and then wash with PBS.

References

1. L. V. Johnson, et al., Localization of mitochondria in living cells with rhodamine 123. PNAS. 1980;77:990-994.
2. C. S. Downes, et al., Novobiocin inhibition of DNA excision repair may occur through effects on mitochondrial structure and ATP metabolism, not on repair topoisomerases. Carcinogenesis. 1985;6:1343-1352.
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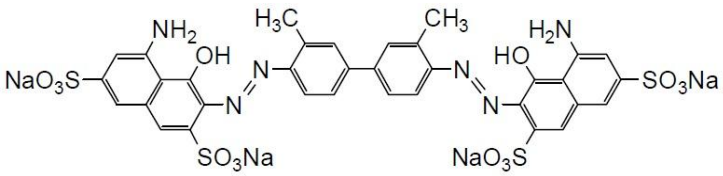
Trypan Blue Cellstain

-Cellstain- Trypan Blue solution - T33190, 5g

3,3'-{[3,3'-Dimethyl-(1,1'-biphenyl)-4,4'-diyl]bis(azo)}-bis(5-amino-4-hydroxy-2,7-naphthalenedisulfonic acid), tetrasodium salt; CAS: 72-57-1; MW: 960.81, C34H24N6Na4O14S4 (Z)

Blackish brown crystalline powder



Trypan Blue is commonly utilized for dead cell staining, in what is called the dye exclusion test. Viable cells are not stained by Trypan Blue. Therefore, dead Trypan Blue-stained cells are easily recognized by microscopy and can be counted using a hematocytometer.Erythrosin B, negrosine, eosin Y, AO and EB are also utilized for this purpose. Though it is hard to detect cells in early to middle stages of apoptosis, Trypan Blue staining is a very simple and widely used method to visualize dead cells.

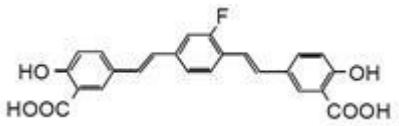
References

1. K. H. Jones, et al., An Improved Method to Determine Cell Viability by Simultaneous Staining with Fluorescein Diacetate-Propidium Iodide. J Histochem Cytochem. 1985;**33**:77-79.

FBS Stain

FBS Solution **CG2370, 100µl**

1-Fluoro-2,5-bis(3-carboxy-4-hydroxystyryl)benzene, 1% w/v DMSO solution; MW: 420.39, C24H17FO6 (L)
Pale yellow to yellowish brown liquid; Absorbance: 0.6-0.85 (around 370nm)



High affinity with β-sheet structure for high detection sensitivity of Amyloidosis

Amyloidosis, a disease which has been identified as a particular disorder by the Japanese Ministry of Health, is an illness that involves an abnormal protein called amyloid that has a β sheet structure, aggregates in fibers, and is deposited on the outside of internal organs and systems, inhibiting the function of those organs and systems. Disorders among many Japanese include immunocytic amyloidosis (AL amylodosis), responsive AA amyloidsis, familial amyloid polyneuropathy (FAP), and dialysis amyloidsis (DRA). It is estimated that there are hundreds of patients throughout Japan. The proteins that cause amyloidosis can be largely divided into two groups: amylids that are deposited in various organs throughout the body (systemic amyloidosis) such as the disorders listed above, and [amyloids that are deposited in a particular organ, such as the brain in the case of Alzheimer's disease (localized amyloidosis). The dye 1-Bromo-2,5-bis(3-carboxy-4-hydroxystyryl)benzene (BSB) has been used for detecting amyloids because of its high affinity with amyloid β peptide (Aβ), the amyloid associated with Alzheimer's disease.

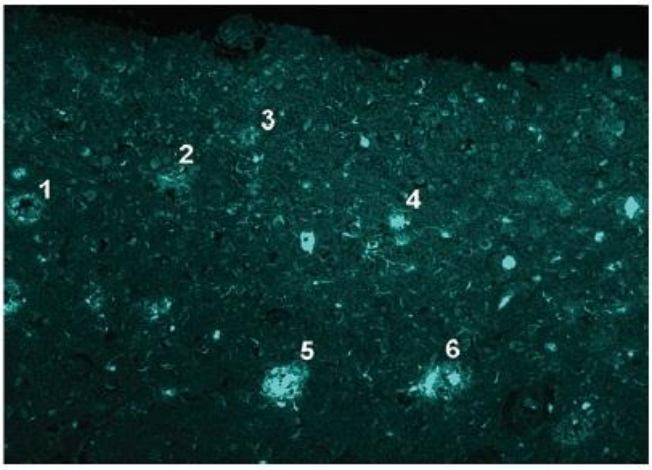


Fig.1 Tissue staining with FSB

A stained image of a segment of the frontal cortex of a patient with Alzheimer's disease. The tissue was fixed with ethanol. The illuminated portions are amyloids. The numbers in the subadjacent slice figures correspond to each senile plaque. (Image was courteously provided by Dr. Higuchi, Dr. Saido, Laboratory for Proteolytic Neuroscience, RIKEN Brain Science Institute.)

Skovronsky confirmed that the dye accumulates in senile plaque of brain tissue of transgenic mice Tg2576 that express the amyloid precursor protein of Aβ(APP) 18 hours after the intravenous injection of BSB.1) Not limited to Aβ, Ando and others have announced that amyloid deposits in various systemic amyloidosis (AA, AL, ATTR, Ascr, Aβ 2M) are stained more sensitively with BSB than Congo red, which is a common dye used for β sheet staining. BSB has twice the fluoresence strength of Congo red. In addition, BSB is not only a staining dye, it is also able to block the amyloid formation FAP's amyloid precursor TTR. Newly developed FSB is also utilized for highly sensitive amyloid staining. This is made possible by the bromine in BSB changing to fluorine and being recovered from the fluorescence quenching caused by the heavy electron effect of bromine. From the results of the stains of Alzheimer patients' brain tissue (Fig. 1) and the heart tissue of AL amyloidosis patients (Fig 2), it appears that FSB detects amyloid deposits better than BSB.

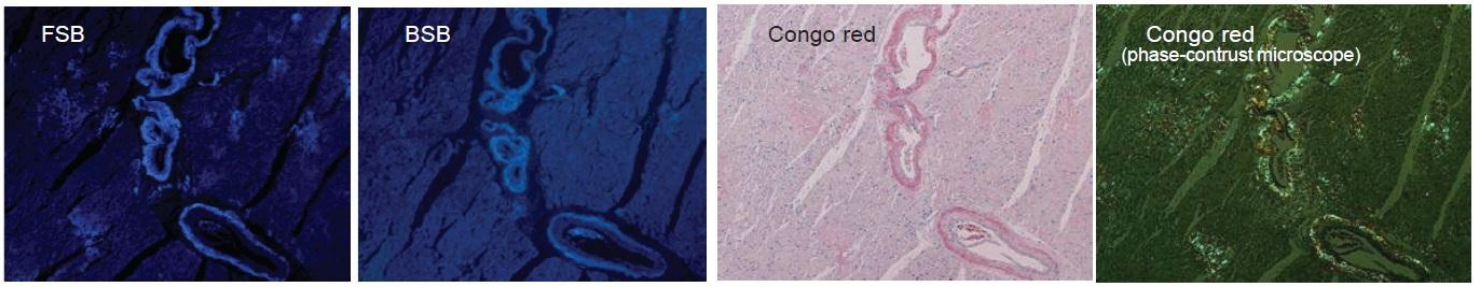


Fig.2 Tissue staining with FSB, BSA, and Congo red

A slice of heart tissue of a patient with AL amyloidosis (Congo red is auburn colored, the illuminated portions of BSB and FSB are amyloids). These are sub-adjacent slices. It is possible to examine finer portions by FSB, and the contrast with the amyloid deposit portions are clear. (Image was courteously provided by Dr. Andoh: Department of Laboratory Medicine, Kumamoto University School of Medicine.)

Protocol

1. Add 50% EtOH to the product and dilute to concentration of 0.01~0.0001% FSB solution.
2. Soak a slice in FSB stain for 30 minutes. After soaking the slice in saturated lithium carbonate, wash with 50% EtOH
3. Detect stained area under UV light (V excitation)

References

1. K. Sato, et al., Fluoro-substituted and 13C-labeled styrylbenzene derivatives for detecting brain amyloid plaques. Eur J Med Chem. 2004;**39**:573-578.
2. M. Higuchi, et al., 19F and 1H MRI detection of amyloid β plaques in vivo. Nature Nurosci. 2005;**8**:527-533.
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4. J. Maeda, et al., Longitudinal, Quantitative Assessment of Amyloid, Neuroinflammation, and Anti-Amyloid Treatment in a Living Mouse Model of Alzheimer's Disease Enabled by Positron Emission Tomography. J Neurosci. 2007;**27**:10957-10968.
5. A. Velasco, et al., Detection of filamentous tau inclusions by the fluorescent Congo red derivative FSB [(trans,trans)-1-fluoro-2,5-bis(3-hydroxycarbonyl-4-hydroxy)styrylbenzene]. FEBS Lett. 2008;**582**:901-906.
6. B. Ji, et al., Imaging of Peripheral Benzodiazepine Receptor Expression as Biomarkers of Detrimental versus Beneficial Glial Responses in Mouse Models of Alzheimer's and Other CNS Pathologies. J Neurosci. 2008;**28**:12255-12267..

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