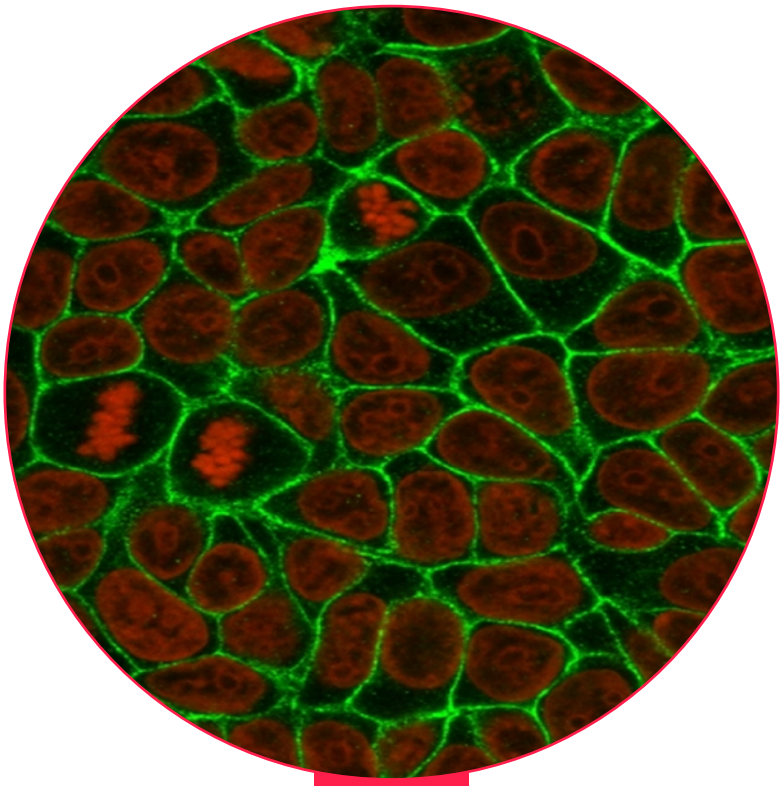
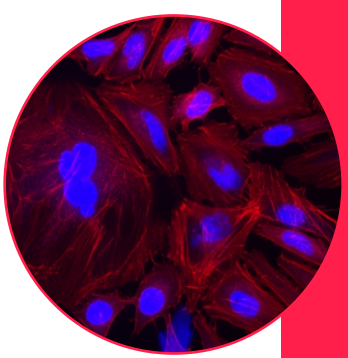
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Cell Fluorescent Staining Technical Manual

A Practical Guide for Fluorescence Staining of Cells





CONTENTS

01 Technical Background

02 Technical Introduction

03 Overview of Cell Fluorescent Dye Technology

1. Cytoskeleton Fluorescent Dyes
2. Subcellular Fluorescent Dyes
3. Cell membrane fluorescent dyes
4. Nuclear Fluorescent Dyes

04 Frequently Asked Questions (FAQ)

I. Technical Background

The cell is the basic structural and functional unit of life. Cell morphology, protein expression, organelle distribution, proliferation, and apoptosis are crucial foundations for research in life sciences, medicine, and pharmaceuticals. Traditional observation methods largely rely on common light microscopy staining techniques such as HE staining and Giemsa staining, which only allow for qualitative observation of the general morphology of cells. They cannot distinguish between live and dead cells, achieve dynamic observation, or perform simultaneous detection of multiple indicators. Furthermore, the results are mostly qualitative, failing to meet the experimental requirements of modern single-cell quantitative analysis and dynamic mechanism research.

To overcome the many limitations of traditional staining techniques and meet the experimental needs of modern cell research for dynamic observation, simultaneous detection of multiple targets, and precise quantitative analysis, fluorescent labeling and microscopic imaging technologies have rapidly developed. Various targeted fluorescent probes are continuously iterating and improving, specifically labeling the cell nucleus, cell membrane, cytoskeleton, and various organelles. Combined with fluorescence microscopy, laser confocal microscopy, and flow cytometry platforms, these techniques effectively overcome the technical bottleneck of traditional staining, which only allows for static qualitative observation. This provides high-precision, multi-dimensional detection methods for cell structure observation, protein localization, and research on cell proliferation and apoptosis mechanisms.

II. Technical Introduction

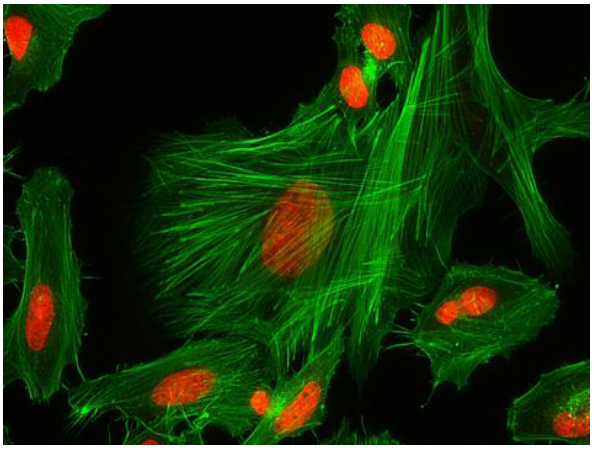
Cellular fluorescence staining is a biological detection technique that utilizes fluorescent probes to bind to specific components within cells (such as DNA, proteins, and organelles), or to react under specific physiological conditions, emitting characteristic fluorescence under excitation light. This fluorescence is then used with equipment such as fluorescence microscopy, laser confocal microscopy, and flow cytometry to observe cell morphology, locate target sites, quantify expression, and analyze cell state. Its core characteristics are high sensitivity, strong contrast, and the ability to simultaneously detect multiple targets, making it a core tool for studying the microscopic mechanisms of cells.

The following section will introduce representative fluorescent probes for the cytoskeleton, organelles, cell membrane, and cell nucleus, according to the different labeled objects, covering their core principles, advantages, and limitations.



1：Cytoskeleton Fluorescent Dyes

1.1 Fluorescein 488-Phalloidin



The cytoskeleton is involved in key physiological processes such as cell morphology maintenance, cell migration, cell division, and adhesion.The most commonly used reagent in the laboratory, and the core reagent that is compatible with the vast majority of fixed cell immunofluorescence experiments, is phalloidin fluorescent dye.Phalloidin is a bicyclic heptapeptide toxin derived from the poisonous mushroom *Amanita phalloides*. It specifically binds to filamentous actin (F-actin) but not to monomeric actin (G-actin), with a binding ratio of approximately 1 molecule of phalloidin to 1 actin subunit. It exhibits no species difference, extremely low background, and is the gold standard probe for staining cytoskeleton microfilaments. Fluorescein 488 Phalloidin uses Fluorescein 488 to label phalloidin, adapted to FITC/488 channel filters, for immobilizing microfilaments in permeabilized samples. It cannot directly stain viable cells(Fluorescein 488-phalloidin cannot cross the cell membrane).

1.2 Fluorescein 488-Phalloidin Principle

(1) Phalloidin embeds itself at the interface between F-actin subunits with extremely high affinity, locking adjacent subunits and thus inhibiting the depolymerization of actin filaments and stabilizing the cytoskeleton structure;
(2) It can also inhibit the ATPase activity of F-actin, preventing ATP from being hydrolyzed into ADP, further maintaining the polymerized state of actin filaments;
(3) Phalloidin can significantly reduce the critical concentration for actin polymerization, promoting the formation and stabilization of F-actin, and is suitable for constructing stable actin filaments in vitro;
(4) Covalently coupled with Fluorescein 488 to form a fluorescent probe, enabling visualization of actin filaments through fluorescence microscopy.

1.3 Advantages of Fluorescein 488-Phalloidin

(1) Extremely high specificity, labeling only polymerized F-actin, superior to actin antibodies; no species bias, suitable for both plant and animal cells;
(2) Small molecular weight, good penetration, suitable for fixed cells and frozen tissue sections;
(3) High signal-to-noise ratio, clearly displaying stress fibers, pseudopodia, filamentous pseudopodia, and cell cortex microfilament structures;
(4) Compatible with multicolor co-localization, can be used in combination with DAPI, red fluorescent secondary antibody, mCherry, etc.;
(5) Extremely simple operation; after sample fixation and permeabilization, labeling can be

(completed in one step by incubating the probe for only 20 minutes.

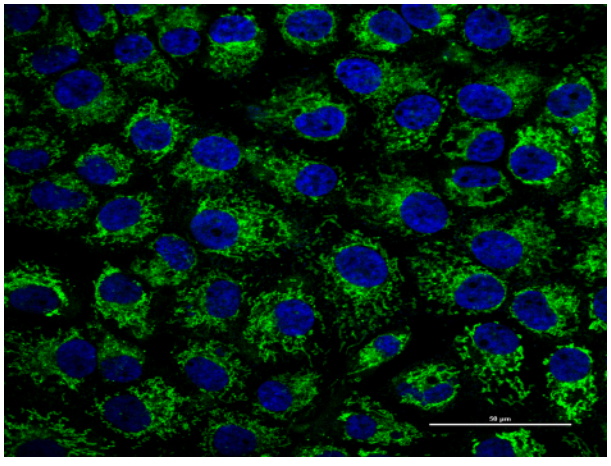
1.4 Precautions for using Fluorescein 488-Phalloidin

(1) Used for sample fixation; cell membranes are impermeable, therefore live cells cannot be directly stained under normal conditions;
(2) Methanol fixation and permeation will damage the microfilament structure; insufficient permeation will result in weak staining or even no signal;
(3) After staining, the sample must be stored in the dark and a mounting medium with anti-quenching properties must be used, otherwise the fluorescence will decay rapidly.

2：Subcellular Fluorescent Dyes

2.1 Mitochondrial Tracker (Green)

Mitochondrial Tracker Green is a cell membrane-permeable cationic cyanine fluorescent probe that accumulates in mitochondria due to its lipophilic cation properties. Mitochondrial localization is independent of mitochondrial membrane potential, allowing labeling of mitochondria even during cell depolarization and early apoptosis. It is suitable for observing mitochondrial morphology, mitochondrial network, and relative mitochondrial mass.
The covalent reactive groups of this probe have limited efficiency under immobilization conditions. After fixation with paraformaldehyde and permeabilization, the fluorescence signal is easily attenuated or even completely lost, resulting in a poor signal-to-noise ratio in fixed cell samples. Therefore, this product is only recommended for staining live cell samples and is not recommended for post-fixation immunofluorescence co-localization experiments.



2.2 Mitochondrial Tracker (Green) Principle

The mitochondrial tracer (green) is a lipophilic cationic cyanine fluorescent dye with high lipid solubility, allowing it to freely penetrate living cell membranes. Its enrichment is primarily independent of mitochondrial membrane potential, relying instead on the partition effect of the lipophilic cation to retain it within the inner mitochondrial membrane, while

simultaneously being covalently anchored to the thiol groups of mitochondrial proteins via chloromethyl groups. This dye exhibits weak fluorescence in aqueous solution, but upon entering the hydrophobic environment of the mitochondria, molecular rotation is restricted, inhibiting non-radiative transitions, thus producing bright green fluorescence.

2.3 Advantages of Mitochondrial Tracker (Green)

(1) Mitochondrial localization is unaffected by mitochondrial membrane potential or depolarization; mitochondria can still be labeled even under apoptosis and oxidative damage models; it accurately reflects the number, morphology, network structure, and fragmentation degree of mitochondria, thus making it suitable for assessing the relative quality of mitochondria.

(2) Nanomolar working concentrations with low cytotoxicity; working concentrations are only 20-200 nM, producing bright fluorescence even at low concentrations; minimal damage to live cells under normal use conditions.

Good cell membrane permeability; simple operation; can be directly added to complete culture medium for incubation without pretreatment; suitable for adherent cells, suspension cells, and primary cells; suitable for fluorescence microscopy, confocal microscopy, and flow cytometry.

2.4 Precautions for using Mitochondrial Tracker (Green)

(1) Only applicable to live cells,fluorescence loss after fixation: Although the molecule contains a chloromethyl reactive group, intracellular covalent coupling efficiency is low, and the dye mainly relies on lipid partitioning for physical retention. After PFA fixation and permeabilization, the dye dissociates extensively, resulting in signal attenuation or even complete disappearance, making it unsuitable for sample fixation and immunofluorescence co-localization.

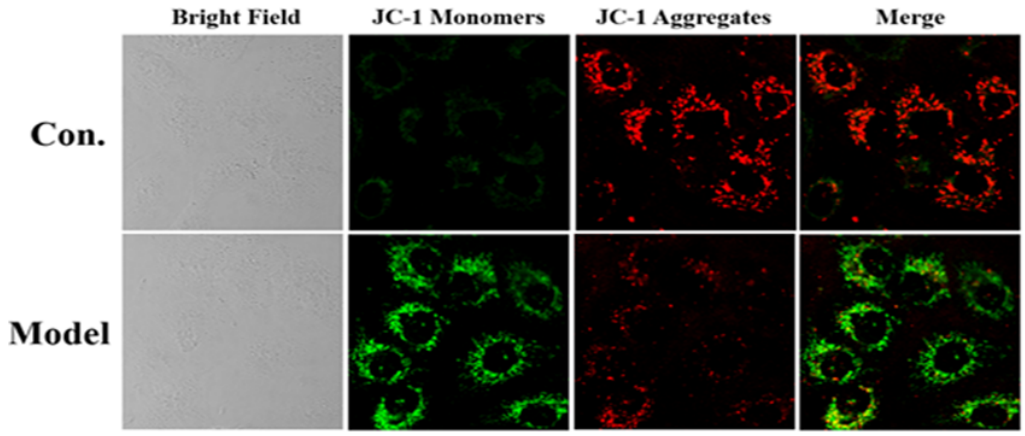
(2) Cannot be used for mitochondrial membrane potential detection: Targeted enrichment is not regulated by membrane potential, and fluorescence intensity cannot reflect membrane potential levels; JC-1 probes are required for membrane potential detection.

(3) Photobleaching occurs: Green cyanine dye itself has limited resistance to photoquenching. During prolonged live cell imaging, the signal easily attenuates, requiring control of laser power and exposure time.

(4) Excessive concentration easily produces non-specific background: When the concentration exceeds the recommended level, the dye will non-specifically bind to other intracellular lipid structures, resulting in diffuse cytoplasmic fluorescence, interfering with mitochondrial imaging results.

2.5 JC-1 Mitochondrial Membrane Potential Probe

JC-1 (5,5',6,6' tetrachloro-1,1',3,3' tetraethylbenzimidazole carbonylcyanine iodide) is a lipophilic cationic cyanine ratiometric fluorescent probe specifically designed for detecting mitochondrial membrane potential (ΔΨm) in live cells. It is a classic tool for assessing mitochondrial function in the early stages of apoptosis. This product is only suitable for live cells; the fluorescence signal is completely lost after fixation and permeabilization, and it cannot be used for fixed samples.



2.6 Principle of JC-1 Mitochondrial Membrane Potential Probe

JC-1 possesses cell membrane permeability and accumulates in the mitochondrial matrix driven by the negative mitochondrial membrane potential. It exists in two reversible forms: monomers and J aggregates. When the mitochondrial membrane potential is high, the probe aggregates at a high concentration within the mitochondria, forming J aggregates and emitting red fluorescence. When the mitochondrial membrane potential decreases and depolarization occurs, the probe is distributed as monomers, emitting green fluorescence. The change in mitochondrial membrane potential can be quantitatively reflected by the red/green fluorescence intensity ratio; a decrease in the ratio indicates mitochondrial depolarization.

2.7 Advantages of the JC-1 mitochondrial membrane potential probe

(1) Ratio-rate detection, reducing systematic errors: Mitochondrial membrane potential is evaluated based on the red/green fluorescence intensity ratio, unaffected by fluctuations in cell number, probe loading efficiency, or instrument exposure parameters. Compared to single-wavelength probes, experimental repeatability is better.

(2) Visually intuitive fluorescence color changes: High membrane potential is indicated by red, turning green upon depolarization. The color change can be directly observed under a fluorescence microscope, suitable for both qualitative imaging and quantitative flow cytometry.

(3) Specific to mitochondrial membrane potential: As a membrane potential-dependent probe, it accumulates in the mitochondrial matrix only when a normal transmembrane potential is present. The fluorescence signal directly responds to changes in ΔΨm, making it suitable for detecting early-stage mitochondrial functional damage during apoptosis.

Simple operation: The probe can be directly added to the complete culture medium for incubation without complex pretreatment. The experimental procedure is simple and mature, and the results are highly comparable.

2.8 Precautions for using the JC-1 mitochondrial membrane potential probe

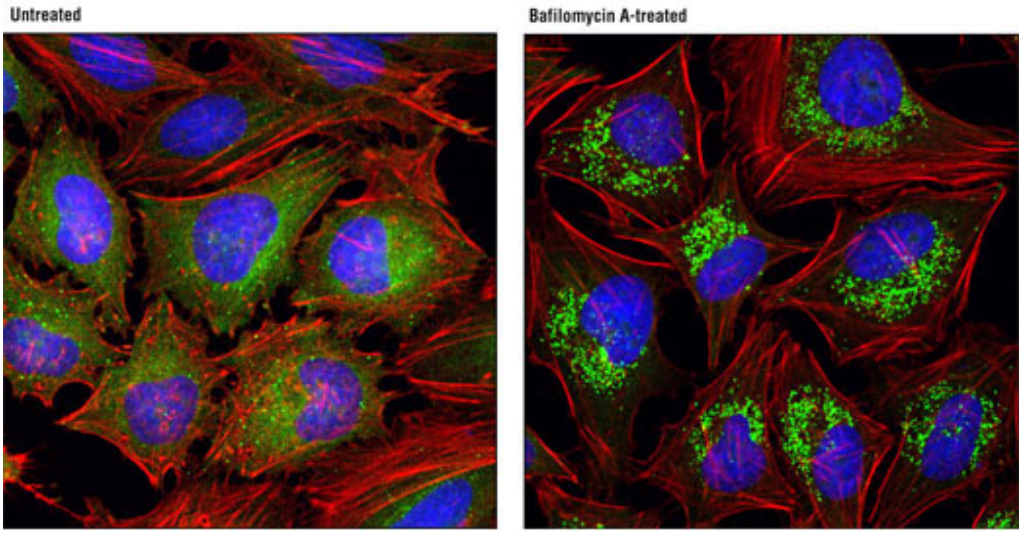
(1) For live cell experiments only. Fixation and permeabilization will completely destroy the detection signal and do not support immunofluorescence co-localization;

(2) A positive control (CCCP mitochondrial uncoupling agent) must be set up to verify the effectiveness of the probe and the experimental system;

(3) Poor water solubility, which easily produces dye precipitation and causes false positive interference.

2.9 Lysosome Tracker (Green)

Lysosome Tracker (Green) probes are fluorescent probes designed for the specific labeling of lysosomes in live cells. They selectively accumulate within acidic lysosomes, enabling specific fluorescent labeling of these organelles. Lysosome Tracker (Green) is suitable for research applications such as observing intralysosomal biosynthesis and related pathogenic mechanisms, monitoring lysosomal number and distribution, and studying the autophagy-lysosome pathway. These probes are intended for staining live cells and are not suitable for staining fixed cells.



2.10 Principle of Lysosome Tracker (Green)

Structurally, Lysosome Tracker (Green) probes consist of a fluorophore linked to a weak base; they freely permeate cell membranes and typically accumulate in spherical organelles. The probe is only partially protonated at neutral pH but becomes enriched within acidic compartments (lysosomes) due to protonation-induced trapping, thereby enabling the selective labeling of lysosomes.

2.11 Advantages of Lysosome Tracker (Green)

(1) Selective labeling of acidic organelles: Selectively stains acidic compartments, such as lysosomes, in live cells.

(2) Low concentration and low toxicity: Effective labeling of live cells can be achieved at nanomolar (nM) concentrations.

(3) Simple procedure: One-step staining; simply add the working solution and incubate.

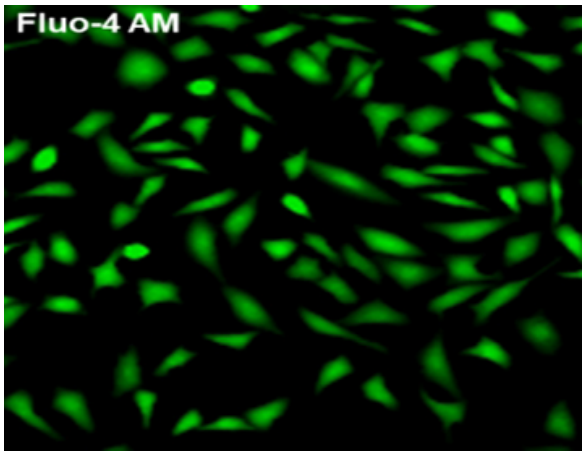
2.12 Precautions for using Lysosome Tracker (Green)

(1) For live-cell experiments only; the probe loses its selectivity for acidic compartments following fixation and permeabilization.

(2) To minimize potential false positives caused by probe overloading, it is recommended to use the lowest effective concentration that yields satisfactory staining.

(3) This probe is a weakly basic compound; prolonged incubation may lead to an increase in lysosomal pH (alkalinization). If the probe is intended for use as a pH indicator, it is recommended to limit the incubation time at 37°C to 1–5 minutes.

2.13 Fluo-4 AM Calcium Fluorescent Probe



Fluo-4 AM is one of the most widely used fluorescent probes for detecting intracellular calcium ion (Ca^{2+}) concentrations; it is a cell-permeable fluorescent dye. Fluo-4 AM is the acetoxymethyl (AM) ester derivative of Fluo-4. The AM form itself exhibits weak fluorescence, but upon entering the cell, it is hydrolyzed by intracellular esterases into Fluo-4 and retained within the cell; it exhibits significantly enhanced fluorescence upon binding to Ca^{2+} (excitation/emission maxima: 494/516 nm).

2.14 Principle of the Fluo-4 AM Calcium Fluorescent Probe

Fluo-4 AM is a cell-permeable esterified precursor; in its AM form, fluorescence is very weak and does not increase with rising calcium ion concentrations. Upon entering the cell, it is cleaved by intracellular esterases to form Fluo-4, which is then retained within the cell. Fluo-4 exhibits strong fluorescence upon binding to Ca^{2+} (increasing more than 100-fold), allowing real-time monitoring of intracellular calcium concentration changes based on fluorescence intensity variations.

Difference from Fluo-3: Fluo-4 AM replaces the chlorine atom in the Fluo-3 AM structure with a fluorine atom. Its maximum excitation wavelength shifts by approximately 12 nm toward the shorter wavelength (494 nm vs. 506 nm), bringing it closer to the 488 nm argon laser line; consequently, its fluorescence intensity under 488 nm excitation is approximately twice that of Fluo-3.

2.15 Advantages of the Fluo-4 AM Calcium Fluorescent Probe

(1) High sensitivity: Fluorescence increases more than 100-fold upon binding to Ca^{2+}, enabling the detection of subtle calcium signal changes;

(2) High brightness: It is brighter than Fluo-3 under identical conditions, with approximately twice the fluorescence intensity of Fluo-3 when excited at 488 nm;

(3) Favorable excitation wavelength: The maximum excitation wavelength is 494 nm, which is closer to the 488 nm argon laser, making it compatible with standard lasers used in confocal microscopy and flow cytometry;

(4) Faster loading: The AM form rapidly permeates the cell membrane, reducing loading time;

2.16 Precautions for Using the Fluo-4 AM Calcium Fluorescent Probe

(1) Fluo-4 AM is highly susceptible to hydrolysis in the presence of water; aliquot and seal the stock solution, keeping it dry, and prepare the working solution immediately before use.

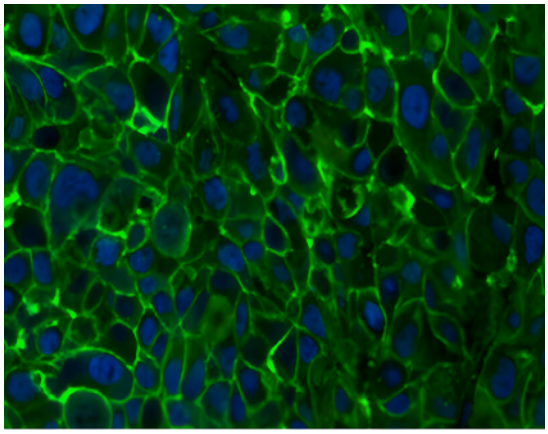
(2) Culture media containing serum possess esterase activity, which can cause premature hydrolysis of Fluo-4 AM into Fluo-4; therefore, thorough washing is required prior to loading.

(3) Culture media containing phenol red can cause a slightly elevated background value; residual medium should be removed as thoroughly as possible before adding the working solution.

3: Cell membrane fluorescent dyes

3.1 DIO (Green Fluorescent Probe for Cell Membranes)

DiO is a green fluorescent, lipophilic carbocyanine and widely used as a lipophilic tracer. It is weakly fluorescent in water but highly fluorescent and quite photostable when incorporated into membranes. It has an extremely high extinction coefficient and short excited-state lifetimes (~1 nanosecond) in lipid environments. Once applied to cells, the dye diffuses laterally within the plasma membrane.



3.2 Principle of DIO (Green Fluorescent Probe for Cell Membranes)

DiO is a long-chain, lipophilic carbonyl cyanine dye with two octadecyl hydrophobic chains. When free in aqueous solution, the molecules aggregate, resulting in weak fluorescence. After the dye inserts into the phospholipid bilayer of the cell membrane, the hydrophobic alkyl chains anchor within the membrane lipids, causing molecular depolymerization and activation of the conjugated fluorescent backbone, producing bright green fluorescence. The dye can diffuse laterally across the same cell membrane, covering the entire membrane. It does not penetrate the transmembrane into the cytoplasm (though prolonged incubation may stain a small amount of intracellular organelle membranes), does not covalently bind to proteins, and anchors itself to membrane structures through hydrophobic interactions.

3.3 Advantages of DIO (Green Fluorescent Probe for Cell Membranes)

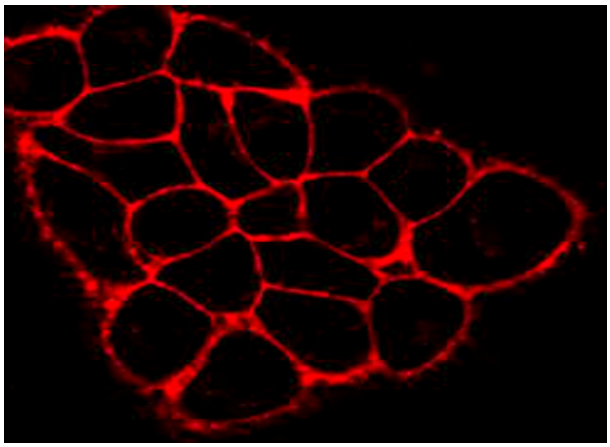
(1) Highly specific for cell membrane targeting, primarily labeling the cytoplasmic membrane.
(2) Low cytotoxicity, suitable for short-term and medium-to-long-term cell tracking.
(3) Fluorescence properties are environmentally sensitive; it exhibits almost no fluorescence in aqueous solution, but fluorescence is significantly enhanced upon binding to the cell membrane with low background.
(4) The spectrum matches commonly used FITC channels in laboratories; it can be used in conjunction with DiI, DiD, and DiR for multicolor cell labeling.

3.4 Precautions for using DIO (green fluorescent probe for cell membranes)

(1) Due to the lipophilic nature of the probe, please avoid using reagents containing glycerol or other organic substances during the experiment, as this will affect the staining effect.
(2) If sample fixation is required, please use a 4% paraformaldehyde solution. Other unsuitable fixatives will result in excessively high fluorescence background.

3.5 CM-DiI (orange-red fluorescent probe for cell membranes)

CM-DiI (Chloromethylbenzamido-DiI, CellTracker CM-DiI) is a chloromethylbenzamido derivative of DiI and belongs to the class of lipophilic indocarbocyanine cell membrane tracing probes. The molecule features two long hydrophobic octadecyl chains and incorporates a reactive chloromethylbenzamido group. Unlike standard DiI, CM-DiI withstands paraformaldehyde fixation, permeabilization, and even paraffin embedding while retaining its fluorescent signal, thereby enabling immunofluorescence and immunohistochemical co-localization experiments following live-cell labeling.



3.6 Principle of CM-DiI (orange-red fluorescent probe for cell membranes)

CM-DiI is a lipophilic indocarbocyanine dye featuring two long hydrophobic octadecyl chains. In aqueous solution, the dye molecules aggregate and exhibit weak fluorescence; however, upon insertion into the phospholipid bilayer of the cell membrane, the hydrophobic alkyl chains anchor within the lipid matrix, causing the molecules to de-aggregate and activating the conjugated fluorescent backbone to produce orange-red fluorescence. The dye can diffuse laterally across the cell membrane, eventually labeling the entire membrane surface. Equipped with a chloromethylbenzoyl reactive group, the dye forms covalent cross-links with the sulfhydryl groups of intracellular proteins after entering the cell, thereby anchoring itself to cellular components.

3.7 Advantages of CM-DiI (orange-red fluorescent probe for cell membranes)

(1) Fixation and permeabilization compatible: Following live-cell staining, the dye supports PFA fixation, Triton permeabilization, and paraffin embedding, as well as subsequent immunofluorescence and immunohistochemistry—a major advantage over standard DiI.

(2) High tracing stability: The dye is covalently anchored and passed to daughter cells during division; the label persists through multiple generations and does not readily transfer to adjacent unlabeled cells.

(3) Low cytotoxicity: It does not significantly affect cell proliferation or survival.

(4) Good photostability: Suitable for long-term imaging, neuronal tracing, and tracking transplanted cells in vivo.

3.8 Precautions for the Use of CM-DiI (Orange-Red Fluorescent Cell Membrane Probe)

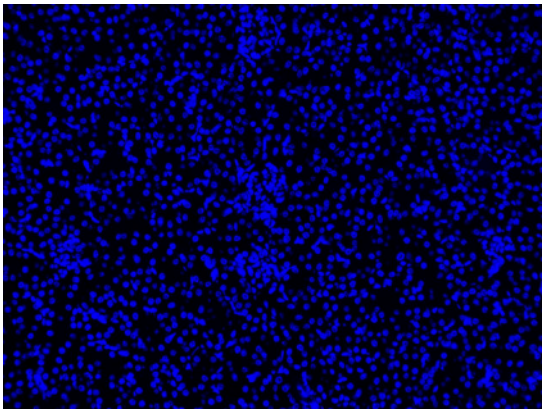
(1) Staining is restricted to live cells: The covalent cross-linking mechanism of CM-DiI relies on

the thiol groups of intracellular proteins; therefore, it cannot directly stain fixed cells or tissues, as covalent coupling cannot occur in fixed samples, resulting in labeling failure.
(2) Preparation requirements for stock and working solutions: This dye has extremely poor water solubility. The solid powder should be dissolved in anhydrous DMSO to create a 1 mM stock solution, which should be aliquoted and stored at -20°C, protected from light, while avoiding repeated freeze-thaw cycles. The working solution must be prepared fresh using pre-warmed complete culture medium; do not dilute the stock solution directly with water or PBS, as this can cause the dye to precipitate and form particulate impurities, leading to non-specific background spotting.

4: Nuclear Fluorescent Dyes

4.1 Hoechst 33342

Hoechst 33342 is a cell-permeable fluorescent DNA dye widely recognized for its ability to bind efficiently to A-T-rich regions within the minor groove of double-stranded DNA. Compared to many other nuclear stains, it features an additional ethyl substituent that enhances its ability to penetrate cell membranes, allowing it to readily cross the plasma membranes of living cells. Consequently, Hoechst 33342 is extensively used in various research fields, including cell biology, immunology, neuroscience, and high-throughput drug screening.



4.2 Principle of Hoechst 33342

Hoechst 33342 is a non-intercalating dye that binds to the DNA minor groove—preferentially at A-T-rich regions—rather than inserting itself between base pairs. Free Hoechst 33342 in aqueous solution exhibits a low fluorescence quantum yield; however, binding to the DNA minor groove triggers several changes:
① Steric constraints restrict molecular motion, reducing non-radiative energy loss;
② Water molecules surrounding the dye are displaced, mitigating water-induced fluorescence quenching;
③ Hydrophobic interactions between the dye's ethyl substituent and consecutive A-T base pairs further stabilize the binding and enhance fluorescence.

The combined effect of these factors results in a 20- to 30-fold increase in fluorescence upon DNA binding.

4.3 Advantages of Hoechst 33342

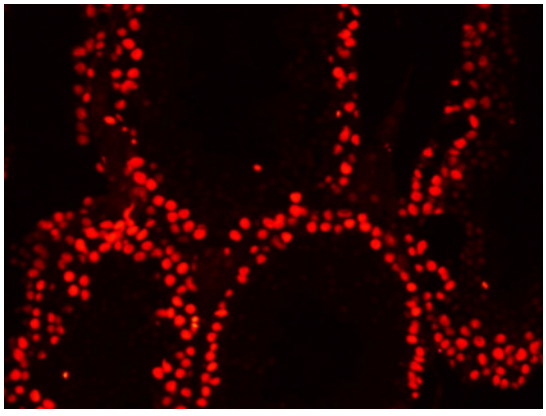
(1)High cell membrane permeability; supports direct staining of live cells without the need for permeabilization.
(2) High nuclear specificity and low cytoplasmic background, resulting in a favorable signal-to-noise ratio for imaging.
(3) Low toxicity in live cells; short-term staining does not significantly affect cell viability.
(4) Suitable for both live cells and fixed samples; compatible with microscopy and flow cytometry platforms.
(5) Excellent spectral compatibility; enables multiplex staining with probes such as FITC, Cy3, and Cy5.

4.4 Precautions for the Use of Hoechst 33342

(1) Some ABC transporter-positive cells efflux the dye, resulting in a diminished staining signal;
(2) As a DNA-binding reagent, it exhibits genotoxicity at high concentrations or with prolonged incubation and is unsuitable for in vivo injection;
(3) It requires UV or 405 nm excitation; photostability is moderate, and the signal is prone to photobleaching during prolonged continuous imaging.

4.5 DRAQ5

DRAQ5 is a far-red fluorescent live-cell DNA dye and is an anthraquinone dye with high affinity for double-stranded DNA. It is a membrane-permeable dye that can label live cells or fixed/dead cells. In flow cytometry, this dye can be used to distinguish between nucleated and non-nucleated cells. Since DRAQ5 can bind to DNA in a stoichiometric ratio, it can also be used to report nuclear DNA content and is suitable for chromosome ploidy and cell cycle analysis. In fluorescence microscopy analysis, it can be used as a nuclear counterstain. DRAQ5 has many applications and is highly compatible with existing instrument platforms and widely used protocols. The main application areas are HCS (high-content screening), cell models, GFP (green fluorescent protein), flow cytometry, and fluorescence microscopy.



4.6 Principle of DRAQ5

DRAQ5 features a protonated tertiary amine side chain and possesses both lipophilic and hydrophilic properties, allowing it to freely permeate intact plasma and nuclear membranes to

stain live cells directly; it is also compatible with fixed cells and tissue samples. The DNA-binding mode of DRAQ5 is concentration-dependent: at standard working concentrations (<5 μM), it primarily binds to the A-T-rich minor groove of double-stranded DNA, whereas at higher concentrations, it undergoes intercalation, inserting itself between DNA base pairs. Electrostatic interactions between the side-chain amino group and the DNA phosphate backbone further stabilize the dye-DNA complex. The dye exhibits high specificity for double-stranded DNA, showing negligible binding to RNA or mitochondrial DNA. In its free state, DRAQ5 is weakly fluorescent; upon binding to DNA, it does not exhibit a significant fluorescence enhancement effect but rather undergoes a spectral red shift. Fluorescence intensity is stoichiometrically proportional to DNA content, making the dye suitable for the quantitative analysis of DNA content.

4.6 Advantages of DRAQ5

(1) Excitation via red light eliminates the need for a UV light source, thereby reducing phototoxicity to live cells;
(2) Exhibits excellent cell membrane permeability, allowing for direct staining of live cells without the need for permeabilization treatments;
(3) Emission occurs in the far-red wavelength range, minimizing spectral overlap with green and orange fluorescent probes and ensuring compatibility in multiplex staining experiments;
(4) Binds to DNA in a stoichiometric manner, making it suitable for DNA quantification and cell cycle analysis;
(5) Suitable for live cells, fixed samples, and tissue sections; compatible with automated high-throughput platforms, with some protocols requiring no washing steps;
(6)Demonstrates superior photostability and resistance to photobleaching compared to Hoechst-series dyes.

4.8 Precautions for Using DRAQ5

(1) It is a DNA-intercalating dye with genotoxicity; it is not suitable for in vivo injection experiments in animals.
(2) It does not distinguish between live and dead cells and cannot serve as a viability dye; cell viability assays should be performed in conjunction with DRAQ7 or PI.
(3) Excessive concentrations or prolonged incubation times can be toxic to live cells; it is unsuitable for long-term live-cell incubation spanning several days.



Frequently Asked Questions (FAQ)

Q1：Issue: Weak or absent fluorescence signal.

A1：Causes: Insufficient dye concentration or dye degradation; excessive cell density or cell viability <70%; incubation time too short; incorrect buffer pH; excessive fixation or permeabilization.

Solutions: ① Prepare fresh stock solution and store at -20°C, protected from light; ② Maintain cell density at 70–80% and viability >90%; ③ Extend incubation time to the recommended upper limit; use 37°C to accelerate staining; ④ Use PBS/HBSS buffer (pH 7.2–7.4); ⑤ Limit fixation time to ⩽15 min and permeabilization (0.1–0.3% agent) to ⩽5 min.

Q2：Issue: High background signal.

A2：Causes: Incomplete washing of residual dye; dye aggregation due to excessive concentration; interference from dead cells or cell debris.

Solutions: ① Wash 3–5 times using buffer supplemented with 0.1% Tween-20; ② Use the lower end of the recommended concentration range; ③ Perform live/dead staining prior to analysis and use flow cytometry gating to exclude dead cells and debris.

Q3：Issue: Severe photobleaching.

A3：Causes: Excitation intensity >50%; exposure time >500 ms; hypoxic environment accelerating fluorescence quenching.

Solutions: ① Reduce excitation intensity to 20–30%; ② Shorten exposure time; ③ Add an anti-quenching agent and seal the sample; ④ Perform imaging at 4°C.

Q4：Issue: Significant cytotoxicity.

A4：Causes: Dye dosage >10 times the recommended concentration; residual DMSO >0.1%; light-induced reactive oxygen species (ROS) generation.

Solutions: ① Strictly adhere to recommended concentrations; optimize via pilot experiments if necessary; ② Ensure final DMSO concentration <0.1% or remove solvent via centrifugation; ③ Protect from light throughout the staining process; consider adding antioxidants.

Q5：Issue: How to handle spectral crosstalk (multicolor labeling)?

A5：Cause: Fluorophore emission peaks overlap by <50 nm; a single excitation beam simultaneously excites multiple fluorescence channels.

Solution: ① Select dye combinations with emission peak separations >100 nm (e.g., DAPI + TRITC + Cy5); ② Use sequential scanning mode; ③ Optimize filters by selecting those with bandwidths <40 nm.

Q6：Issue: Why is the dye localization abnormal?

A6：Cause: Changes in mitochondrial membrane potential; non-specific binding due to excessively high dye concentration.

Solution: ① Use probes that are independent of membrane potential (e.g., MitoTracker Green FM); ② Reduce dye concentration and limit incubation time to ⩽30 min; ③ Co-stain with specific antibodies to verify localization.

Q7：Issue: How can dye precipitation/aggregation be resolved?

A7：Cause: Excessively high stock solution concentration; solvent evaporation; poor dye solubility in the culture medium.

Solution: ① Prepare stock solutions at 1–10 mM using high-quality anhydrous DMSO; ② Aliquot and freeze, avoiding repeated freeze-thaw cycles; ③ Add a solubilizing agent and pre-warm the culture medium to 37°C.

EnkiLife
Cell Fluorescent Dye Series Product Recommendations

EnkiLife focuses on the development of fluorescent labeling tools for cells, establishing a comprehensive product portfolio that includes fluorescent dyes for the cytoskeleton, subcellular structures, cell membranes, and nuclei. The spectral range spans the entire spectrum—from blue, green, orange-red, and red to near-infrared—supporting both live-cell real-time imaging and fixed-sample staining. These tools cater to diverse experimental needs, ranging from single-marker observation to multicolor co-localization, single-cell quantification, and high-throughput drug screening, thereby providing stable and reproducible visualization tools for research in cell biology, cancer biology, neuroscience, and pharmaceutical screening.

Product Advantages

1. High Specificity and Sensitivity

• **Specific Binding:** Cellular fluorescent dyes bind specifically to target cellular structures or molecules—such as cell membranes, nuclei, mitochondria, and lysosomes—ensuring signals originate solely from the target area with minimal background fluorescence.

• **High Sensitivity:** Fluorescence signal intensity is directly proportional to the concentration or abundance of the target molecule, allowing for detection by high-sensitivity microscopes or imaging systems even at low concentrations.

2. Low Toxicity and High Stability

• **Low Cytotoxicity:** Optimized chemical design ensures minimal impact on cellular physiological functions, making the dyes suitable for long-term live-cell imaging.

• **High Stability:** Fluorescence signals exhibit exceptional stability across various experimental conditions (e.g., varying pH, temperature, and light exposure) and are resistant to photobleaching.

3. Diverse Fluorescence Wavelengths

• **Broad Wavelength Range:** Cellular fluorescent dyes cover the spectrum from visible to near-infrared light (400–750 nm), meeting diverse experimental needs ranging from single-color to multiplex labeling.

• **Low Background Fluorescence:** Near-infrared dyes (e.g., 690–750 nm) offer reduced background fluorescence and superior tissue penetration, making them ideal for *in vivo* imaging.

4. Ease of Use and High Compatibility

• **Ease of Use:** Most cellular fluorescent dyes are simple to use, requiring minimal staining steps and no complex sample pretreatment.

• **High Compatibility:** They are highly compatible with common cell fixatives, permeabilization agents, and other fluorescent markers, allowing for seamless integration into various experimental workflows.

Product Categories	Cat.No	Product Name	Ex/Em
Cell skeleton fluorescent dyes	RA20015	Rhodamine-Phalloidin	546/575 nm
	RA20010	Fluorescein 488-Phalloidin	490/515 nm
	RA20011	Fluorescein 555-Phalloidin	555/565 nm
	RA20012	Fluorescein 594-Phalloidin	590/617 nm
	RA20013	Fluorescein 633-Phalloidin	630/650 nm
	RA20014	Fluorescein 680-Phalloidin	681/698 nm
Subcellular structure fluorescent dyes	RA20021	Mitochondrial Tracker (Green)	490/523 nm
	RA20020	Mitochondrial Tracker (Red)	579/599 nm
	RA20025	4-Di-1-ASP (Mitochondrial Fluorescent Probe)	474/606 nm
	RA20024	DASPEI (Mitochondrial Fluorescent Probe)	461/589 nm
	RA20022	JC-1 (Mitochondrial Membrane Potential Probe)	485/535 nm (Monomers), 550/600 nm (J-Aggregates)
	RA20028	Lysosome Tracker (Green)	504/511 nm
	RA20027	Lysosome Tracker (Red)	577/590 nm
	RA20026	Lysosome Tracker (DeepRed)	649/665 nm
	RA20033	Fluo-4 AM, Calcium Ion Fluorescent Probe	494/516 nm
	RA20030	Dihydroethidium(Hydroethidine;DHE)	355/420nm (Unoxidized) 518/610nm (After oxidation)
	RA20088	Fluo-20 AM Calcium Probe(Green)	495/516 nm
	RA20034	Fluo-3 AM, Calcium Ion Fluorescent Probe	506/526 nm
	RA20031	Fluo-3 AM (2mM), Calcium Ion Fluorescent Probe	506/526 nm
	RA20032	Fluo-4 AM (2mM), Calcium Ion Fluorescent Probe	494/516 nm
	RA20087	Fluo-8 AM Calcium Probe(Green)	495/516 nm

Product Categories	Cat.No	Product Name	Ex/Em
Cell membrane fluorescent dyes	RA20005	DiO Membrane Probe (Green)	484/501 nm
	RA20004	DiI Membrane Probe (Orange-Red)	549/565 nm
	RA20003	DiD Membrane Probe (Red)	644/663 nm
	RA20006	DiR Membrane Probe (Near-Infrared)	748/780 nm
	RA20008	CM-DiD Membrane Probe (Red)	644/663 nm
	RA20002	Dilinoleyl DiI Membrane Probe (Orange-Red)	549/565 nm
	RA20007	CM-DiI Membrane Probe (Orange-Red)	549/565 nm
	RA20019	Enhanced DiO Membrane Probe (Green)	484/501 nm
	RA20001	DiA Membrane Probe (Green)	456/590 nm
Nuclear fluorescent dyes	RA20036	DAPI Nuclear Dye	360/460 nm
	RA20035	DAPI Nuclear Staining Solution (Ready-to-use)	360/460 nm
	RA20039	Hoechst 33342 Live Cell DNA Dye	350/461 nm
	RA20037	Hoechst 33342 Nuclear Staining Solution (Ready-to-use)	350/461 nm
	RA20040	Hoechst 33258 Live Cell DNA Dye	346/460 nm
	RA20038	Hoechst 33258 Nuclear Staining Solution (Ready-to-use)	346/460 nm
	RA20041	Propidium Iodide (PI)	535/617 nm
	RA20067	DRAQ5 Live Cell DNA Staining Solution	647/681 nm
	RA20016	5(6)-CFDA SE Live Cell Fluorescent Probe	492/517 nm
	RA20017	6-CDCFDA SE Live Cell Fluorescent Probe	504/529 nm
	RA20042	dsDNA Quantification Reagent (Green)	480/520 nm
	RA20068	High Sensitivity dsDNA Quantification Kit	485/530 nm

Thank you
for choosing
us!