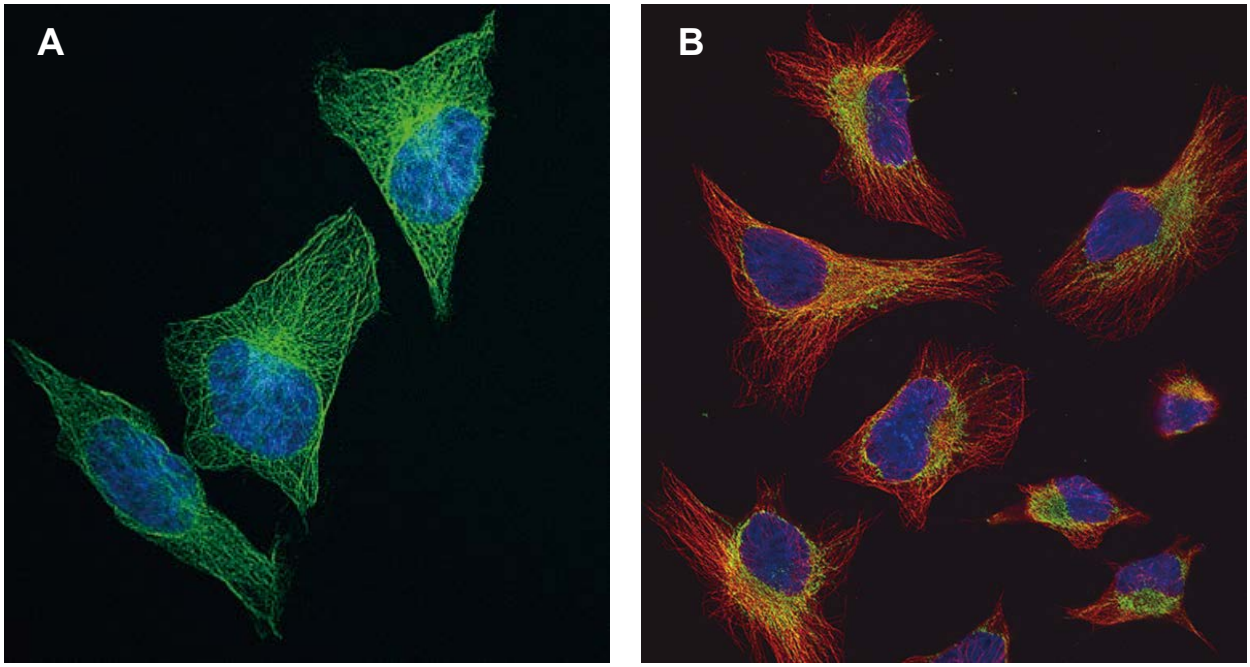
LIFE SCIENCE

Cell Biology Reagents

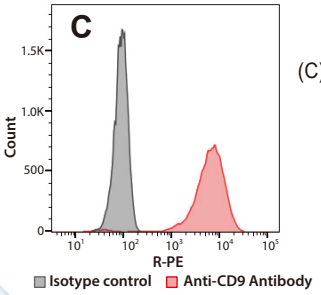
Fluorescent Cell Stains

Applications



(A) The HeLa cells were incubated with properly diluted primary antibody (Mouse Anti α-Tubulin IgG) and were further incubated with Goat Anti-Mouse IgG Biotin Conjugate [G0387] and Streptavidin FITC Conjugate [S0966] (green fluorescence). And then the nuclei was stained with DAPI·2HCl [A2412] (blue fluorescence). (Laser Scanning Microscope: Olympus FLUOVIEW FV 3000)

(B) The nuclei of HeLa cells was stained with Bisbenzimide H 33258 [H1343] (blue fluorescence). α-Tubulin was stained with anti-α-tubulin antibody and Goat Anti-Mouse IgG Biotin Conjugate [G0387] and Streptavidin R-PE Conjugate [T3885] (red fluorescence). Mitochondria was stained with primary antibody and Goat Anti-Rabbit IgG FITC Conjugate [G0452] (green fluorescence)**. (Laser Scanning Microscope: Olympus FLUOVIEW FV 3000)



(C) The HeLa cells were incubated with Mouse Anti-CD9 Antibody (red line) or Mouse IgG2aκ isotype control (black line). Subsequently, both were stained with Goat Anti-Mouse IgG Biotin Conjugate [G0387] and Streptavidin R-PE Conjugate [T3885]. (Flow cytometer: Sysmex RF-500)

**Please refer to our product page for staining procedure.
R-PE/FITC-labeled anti-Mouse IgG or anti-Rabbit IgG antibodies and streptavidins can be used for fluorescence immunostaining and flow cytometry.

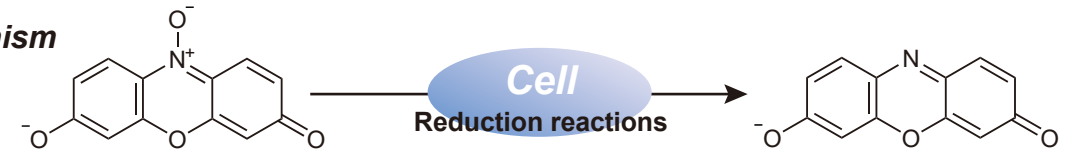
Goat Anti-Mouse IgG FITC Conjugate (Green Fluorescence)	0.1mg/vial	[G0406]
Goat Anti-Mouse IgM FITC Conjugate (Green Fluorescence)	0.1mg/vial	[G0453]
Goat Anti-Rabbit IgG FITC Conjugate (Green Fluorescence)	0.1mg/vial	[G0452]
Streptavidin FITC Conjugate (Green Fluorescence)	0.1mg/vial	[S0966]
Goat Anti-Mouse IgG R-PE Conjugate (Red Fluorescence)	0.1mg/vial	[G0569]
Goat Anti-Rabbit IgG R-PE Conjugate (Red Fluorescence)	0.1mg/vial	[G0577]
Streptavidin R-PE Conjugate (Red Fluorescence)	0.1mg/vial	[T3885]
Goat Anti-Mouse IgG DTBTA-Eu^{3+} Conjugate (Red Fluorescence)	0.1mg/vial	[G0505]
Goat Anti-Rabbit IgG DTBTA-Eu^{3+} Conjugate (Red Fluorescence)	0.1mg/vial	[G0506]
Streptavidin DTBTA-Eu^{3+} Conjugate (Red Fluorescence)	0.1mg/vial	[S0993]
DAPI·2HC (Blue Fluorescence)	5mg	[A2412]
Bisbenzimide H 33258 Hydrate (Blue Fluorescence)	25mg	[H1343]

*Some products are unavailable in the U. S. and China.
*The high-sensitivity detection of DTBTA-Eu^{3+} labeled probes requires time-resolved fluorometry.

Cell Proliferation Assay Reagents

Resazurin (Ready-to-use solution) [for Cell proliferation assay] 25mL [R0195]

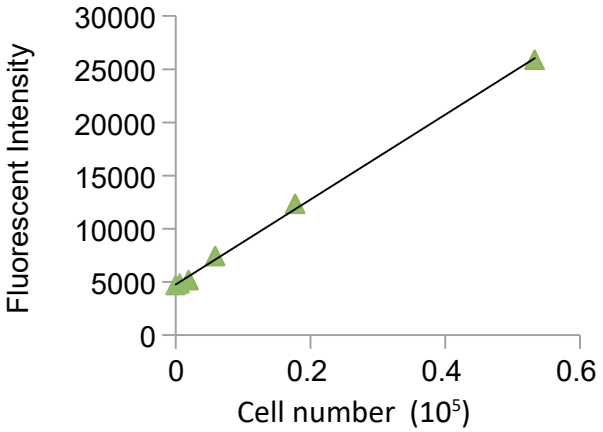
Mechanism



Ex:540-570nm, Em:590 nm

Resazurin can be used quantitatively determine cell proliferation, viability, and cytotoxicity.
Resazurin, when added to viable cells, is reduced by the cellular enzymatic or chemical reactions converting blue/non-fluorescent resazurin to highly fluorescent resorufin.
The assay is simple to perform since the indicator is water-soluble and has low toxicity, thus eliminating the washing/fixing and extraction steps required in other commonly used cell proliferation assays.

Cell viability assay



Application

1. Add **R0195** at a volume equal to 10% of the cell culture media volume.
2. Return cells to the incubator and continue the incubation for 2-24 hours*.
3. Measure the fluorescent intensity using 540-570 nm excitation and 590 nm emission wavelengths. Absorbance can be measured using a spectro-photometer set at 570 nm.

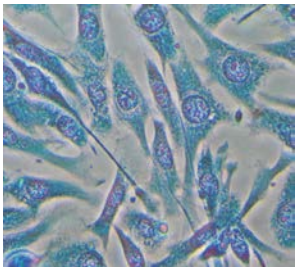
Resazurin may be added at any time point during the culture period. For measurement of cell proliferation, it is best to add resazurin during the log phase of growth.

Cell Staining Dyes

Methylene Blue Solution (Methanol Solution) [for Cell Staining] 100mL [M2392]

Application

(1) Culture cells in a 6-well plate.
(2) Remove medium from the plate and wash it with PBS(-) twice.
(3) Remove PBS(-) from it, add 1mL of **M2392** and stain cells for 15 minutes.
(4) Remove **M2392** from it and wash it with deionized water twice.



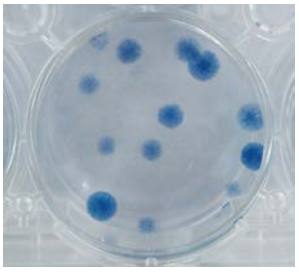


Figure. NIH/3T3 cells stained by the above method

Please adjust staining time and volume according to cells. Because some cells need to be fixed separately, preliminary tests should be performed.

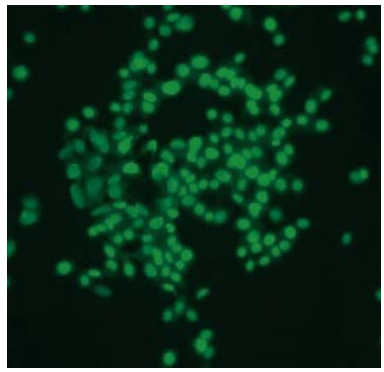
Acridine Orange Solution [for Cell Staining] 5mL [A3396]

Acridine orange is a nucleic acid staining dye that is used to identify dead cells. It intercalates with the double-stranded DNA base pairs at a ratio of 1:3 and is capable of emitting green fluorescence (Ex: 500 nm, Em: 520 nm). It also emits red fluorescence (Ex: 460 nm, Em: 650 nm) when bound to RNA or single-stranded DNA. Since acridine orange is mutagenic, this product is supplied as a solution that prevents it from splashing during weighing.

Application: The method of staining cells by A3396

1. Remove the medium from the culture plate and wash the cells twice with cold PBS(-). Remove the PBS(-).
2. Add PBS(-) and Acridine Orange solution [A3396] (1/50th of the volume of the added PBS(-)) and incubate for 15 minutes.
3. Remove the staining solution and wash the cells twice with PBS(-).
4. Add PBS(-) and observe the cells under a fluorescence microscope.

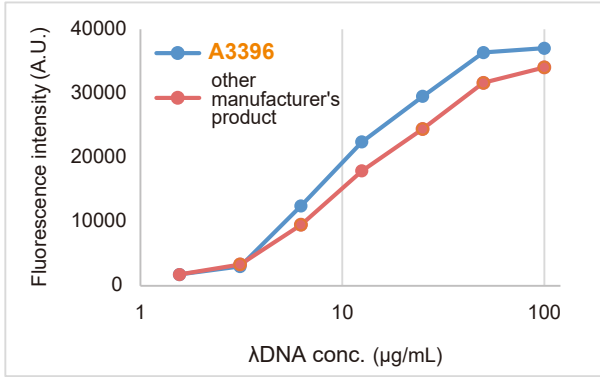
Please adjust the staining duration and the volume of the solution according to the cell density.
Some cells may require prior fixation; therefor optimization of the protocol according to your need is recommended.



NIH3T3 cells stained by A3396

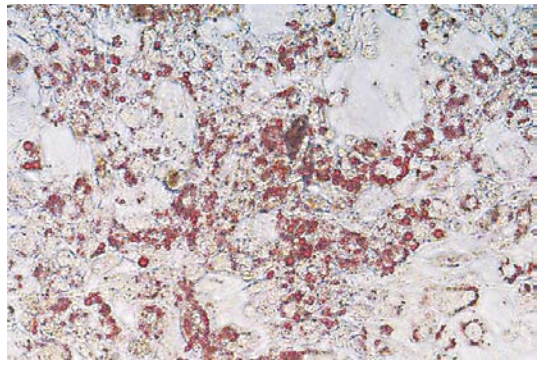
λDNA was stained using either the acridine orange solution [A3396] or the other manufacturer's product, and the fluorescence measured from two experiments was compared (Ex:500nm, Em:520nm).

The results indicated that A3396 stained better than the other manufacturer's product.



Oil Red O [for Biochemical Research] 25g [O0483]

Oil red O is a diazo dye which has been widely used for staining fat cell and lipids since long ago. This lysochrome (fat-soluble dye) red dye can be used for staining of neutral triglycerides and low polar lipids. Oil Red O staining is done on fresh or frozen samples, since alcohol fixation removes lipid. Its usage is simple, and requires only washing after adding the staining solution, which makes it easy to identify lipids visually. Furthermore, accumulation of lipids can be quantified by eluting the dye using isopropanol after staining and measuring the absorbance.



10 days later of addition of media for differentiation to 3T3-L1 cell and then stained by 1mg/mL of O0483.

Extraction Buffer for Mammalian Cells

RIPA Buffer (Ready-to-use) [for Protein extraction] 100mL [R0246]

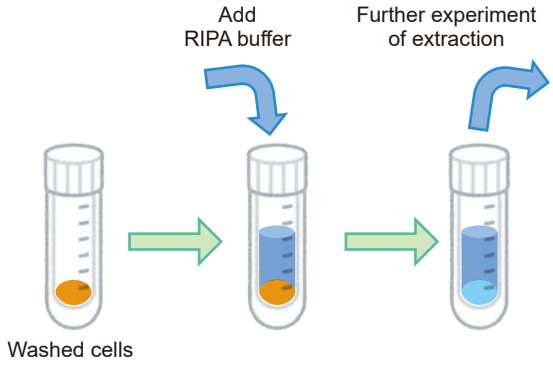
This product is supplied as a ready-to-use solution for the lysis of the cultured mammalian cells. Proteins can be extracted by adding this buffer [R0246] to the cells and the extract can be used directly for further analysis such as western blotting. This product does not include protease inhibitors. Please add a protease inhibitor cocktail, if necessary.

Application

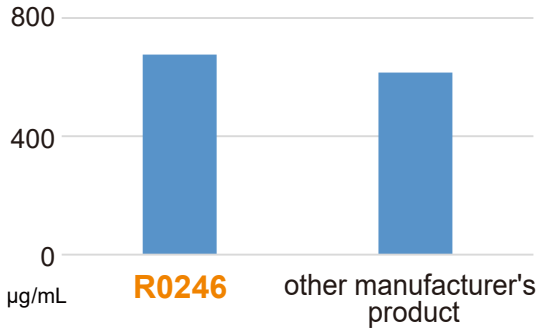
Add the following protease inhibitors to RIPA buffer [R0246].

Leupeptin	10 µg/mL
Pepstatin A	1 µg/mL
Aprotinin	3 µg/mL
AEBSF	1 mM

1. Wash the cultured mouse myeloma-derived cell sp2/0 twice with PBS.
2. Remove PBS and add 200 µL of either cold RIPA buffer [R0246] containing protease inhibitors or the other manufacturer's RIPA buffer containing the same protease inhibitors to 1.0 x 10^6 cells.
3. Incubate the cells for 15 minutes on ice.
4. Centrifuge the cells at 10,000 x g for 10 minutes at 4 °C
5. Measure the protein concentration of the supernatants.
6. Analyze the supernatants using western blotting.



Extracted Protein Concentration



Western Blotting

The extracts were transferred to a PVDF membrane after electrophoresis.
Anti-β actin antibody was used for detection.
Equal or better detection was observed than that of the other manufacturer's product.

For further information please refer to our website at www.TCIchemicals.com. ▶▶▶ cell biology

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