

Stem Cell CDy1 Dye

Catalog No: 14001

Format: 50 µl

Chemical Properties: C₃₂H₄₀CIN₃O₂

Stem Cell CDy1 Dye is supplied as a 50 µl stock solution in DMSO.

Fluorescent Properties:

Excitation: 544 nm

Emission: 577 nm

Quality Control: The following protocol is designed to be used with Active Motif's Stem Cell CDy1 Dye for selective staining of live stem cells. The CDy1 dye can be used for staining and imaging or FACS analysis of ESCs, iPSCs and various stem cell types.

Storage and Guarantee: Store at -20 °C. We recommend making 10 µl aliquots upon first use to avoid repeated freeze/thaw cycles. This product is for research use only and is not for use in diagnostic procedures. This product is guaranteed for 6 months from date of arrival.

Protocol: Stem Cell CDy1 Dye

If the cells are to undergo immunostaining for additional stem cell markers, the cells should be stained with CDy1 first, prior to fixation of the cells with 4% paraformaldehyde and permeabilization with 0.1% Triton X-100. The CDy1 dye is specific for live stem cells. Cells that are dead or damaged will also be stained, independent of their differentiation status, and may contribute to background signal.

For additional protocol details, please refer to the following reference:

Kang, N.Y. et al. (2011) *Nat. Protoc.*, 6: 1044-1052.

Optimization may be required to determine ideal staining and destaining times for your specific stem cell line and culture conditions.

Preparation of the CDy1 Buffer Solution

1. Dilute the CDy1 dye stock 1:40 with PBS by adding 10 µl CDy1 dye to 390 µl PBS.

Note: As the Stem Cell CDy1 dye is dissolved in DMSO, it is recommended to make a negative control solution using the same volume of DMSO as that used for the dye solution. This negative control solution should be added to samples as an unstained control to determine if there are any artifacts caused by the addition of the solvent to the cells.

2. Store the CDy1 buffer solution protected from light at 4°C. The diluted dye solution is stable for 1 month at 4°C.

Staining and Washing of the Stem Cells

1. Grow stem cells in the established culture conditions.
2. Prepare pre-warmed (37°C) culture medium and PBS. We recommend a volume of 2 ml per well of a 6-well plate. For staining and washes of a 6-well plate you will need to pre-warm 4 ml per well of culture medium and 6 ml per well of PBS. Please adjust volumes as needed for your culture conditions.
3. Dilute the CDy1 buffer solution prepared above 1:100 with pre-warmed culture medium by adding 20 µl of the CDy1 buffer solution to 2ml pre-warmed (37°C) culture medium. (You may want to try a range of dilution factors to determine the optimal staining concentration for your sample. We suggest trying 1:50, 1:100 and 1:500). For control wells, dilute the negative control solution 1:100 in pre-warmed culture medium (Prepare negative control solutions for each dilution factor tested).
4. Remove the existing culture medium and add the CDy1 staining solution or negative control solution to the stem cells. Immediately mix to avoid focal toxic effects of DMSO.
5. Incubate the cells at 37°C in the cell culture incubator with 5% CO₂ for 1 hour.
6. Wash the cells 3 times (2 ml per well of a 6-well plate) with pre-warmed PBS. (Alternatively, washing can be done using warmed cell culture medium).
7. Destain the cells by adding 2 ml pre-warmed culture medium to each well and incubate the cells at 37°C in the cell culture incubator with 5% CO₂ for 3 hours. (Destaining time can be increased to reduce background signal).

Imaging by Fluorescence Microscopy

1. Use a TRITC / Cy3 filter set to take images with a fluorescent microscope.
2. The CDy1 dye has the following spectral properties: Maximum excitation = 544 nm, Maximum emission = 577 nm

Imaging by Flow Cytometry and FACS sorting

1. Use established procedures like trypsin treatment to prepare a single cell suspension of the stained cells.
2. A concentration of 2 x 10⁵ cells per ml solution can be used for FACS analysis.
3. The Stem Cell CDy1-positive cells can be detected and sorted using a 488 nm laser and a PE-Texas Red filter.

Troubleshooting Guide for Stem Cell CDy1 Dye

High background signal detected on feeder layer

1. Nonspecific staining due to unhealthy feeder cells. Prepare the feeder layer again and use as early as possible to avoid dead or damaged cells.
2. Prolonged incubation time, such as overnight incubation with CDy1. Increase the destaining time to help reduce background.

Weak or no staining detected

1. The stem cells have differentiated. Maintain the stemness of the cells by careful culturing.
2. For iPSCs, this can be the result of low reprogramming efficiency.
3. Increase the staining time to longer than 1 hour to enhance the intensity of the stain.