

NEW

# Stem Cell CDy1 Dye

live cell stain for identification and imaging of stem cells

Active Motif's new Stem Cell CDy1 Dye was developed in collaboration with scientists at the Agency for Science, Technology and Research (A\*STAR) in Singapore as a live cell imaging probe that is selective for stem cells. CDy1 offers a faster, higher throughput and more cost-effective way to identify and isolate pluripotent stem cells and other stem cell types, including neural and cancer stem cells, compared to conventional methods. The cell-permeable fluorescent dye is easy to use. Simply dilute CDy1 in media, apply to cells, incubate for 1 hour and wash. Because CDy1 is non-toxic and non-permanent, it eliminates the need to sacrifice precious stem cell samples for testing. This provides a significant advantage over other methods such as immunostaining, alkaline phosphatase or reporter assays that compromise cellular integrity through fixation or cell manipulation. As CDy1 does not impact growth, morphology, or differentiation, it is ideal for FACS sorting and enrichment of stem cells and enables additional experiments or counterstaining procedures to be performed following CDy1 staining.

## Why is Stem Cell Identification Important?

Stem cells are unique among biological cell types in that they are unspecialized cells that are capable of self-renewal through cell division and also possess the ability to differentiate into a multitude of cell types (Figure 1). The unique properties of self-renewal and potency make stem cells invaluable research tools for studies of development, aging, regeneration and cancer. Stem cells have also been targeted as potential therapeutics for certain diseases. As the use of stem cells continues to expand in these areas of research, selective tools for the accurate identification and isolation of stem cells are becoming more critical.

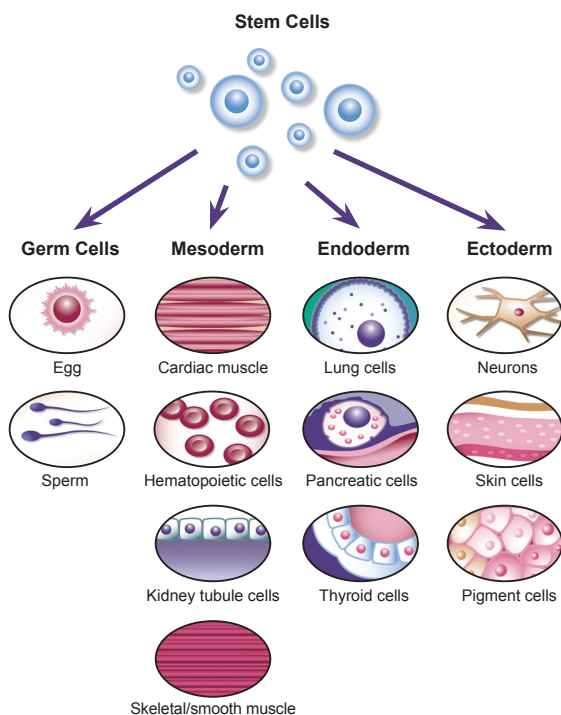


Figure 1: Fate of Stem Cells.

## Novel Stem Cell Dye for Imaging of Live Cells

Active Motif's **Stem Cell CDy1 Dye** is a non-toxic, non-permanent live cell stain designed for the detection and imaging of stem cells by FACS or fluorescent microscopy. CDy1 was developed in collaboration with researchers at A\*STAR who performed screens to identify novel small-molecule fluorescent probes specific for pluripotent stem cells.<sup>4,5</sup> CDy1 was discovered in these screens as a cell-permeable dye that could selectively stain live embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs). CDy1 is now known to also selectively stain cancer stem cells<sup>1</sup> and neural stem cells.<sup>2</sup> The unique chemical and spectral properties and ease of use of CDy1 make it the ideal tool for identifying and selecting stem cells from differentiated or feeder cells in mixed cultures and for screening of iPSCs during reprogramming. The CDy1 method requires no cell sacrifice and has no effect on biological function, giving you more options for analyzing your precious stem cell samples.

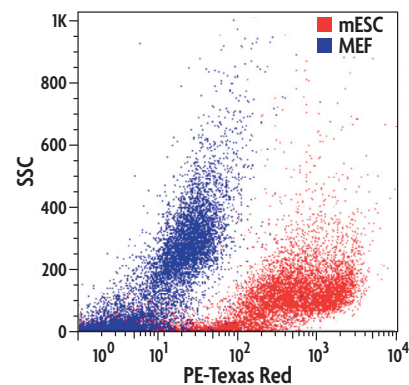


Figure 2: FACS Data of CDy1 Staining on mESCs and MEFs.

Flow cytometry dot-plot image overlay of pure populations of Stem Cell CDy1 stained mouse ESCs (mESC) in red and mouse embryonic fibroblasts (MEF) in blue. The cells were analyzed using side scatter (SSC) and a PE-Texas Red channel. CDy1 enables clear identification and isolation of embryonic stem cells.

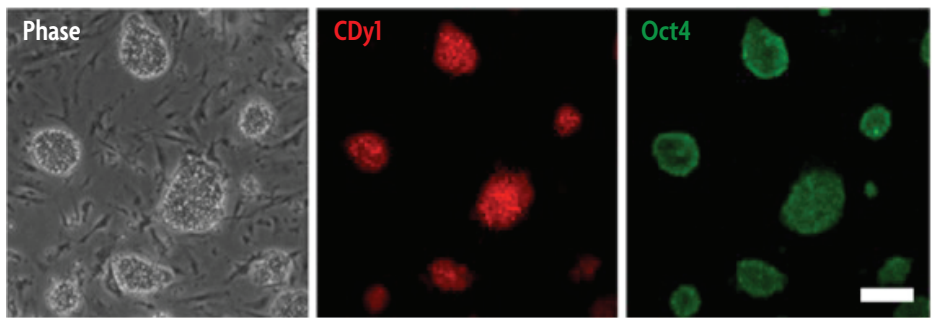
## Why Use the Stem Cell CDy1 Dye?

CDy1 offers a fast, simple and minimally invasive cell staining method to identify and isolate live stem cells from feeder cells (Figure 2, opposite page) or differentiated cells in mixed cultures, and for screening iPSC colonies during early stage reprogramming.<sup>4</sup> The unique properties of the CDy1 dye bypass many of the limitations presented by conventional methods. With CDy1's ability to stain living cells, there is no longer a need to sacrifice precious stem cell samples. Cells that have been stained will have the same growth rate, morphology and differentiation capacity as unstained stem cells. Because cellular integrity is preserved, further experimental or immunostaining procedures can be performed following CDy1 staining (Figure 3).

A major advantage of CDy1 is its ease of use. Simply dilute the Stem Cell CDy1 Dye in culture media and add to your cells. Following a 1 hour incubation and 3 hour destaining, cells are washed and prepared for imaging by fluorescence microscopy or FACS analysis. The spectral properties of CDy1 enable detection of stained cells by fluorescent microscopy using common TRITC or Cy3 filter sets, while a 488 nm laser and a PE-Texas Red filter are used for analysis by flow cytometry. The ease of use of CDy1 makes it conveniently adaptable to higher throughput processing. Another advantage is that CDy1 eliminates the need to generate a reporter system to screen for stem cell marker expression. CDy1 has also been shown to select for iPSCs at the early stages of reprogramming and identifies iPSC colonies at much earlier timepoints than genetic reporter systems.<sup>4</sup>

### CDY1 ADVANTAGES

- Non-toxic & non-permanent
- No fixation or sacrifice of cells required
- Imaging of cells by FACS or fluorescence
- Uses common filter sets (Ex. 544 nm, Em. 577 nm)
- Identifies iPSCs earlier than genetic reporter systems
- Simple & rapid staining protocol



**Figure 3: CDy1 Staining Coincides with Expression of Oct4 Stem Cell Marker.**

Mouse embryonic stem cells (mESCs) grown on a mouse embryonic fibroblast (MEF) feeder layer were stained without fixation with Stem Cell CDy1 Dye. CDy1 staining was visualized by fluorescent microscopy. The cells were then fixed in 4% paraformaldehyde, stained with Oct4 antibody and visualized with FITC-conjugated secondary antibody. The images show that mESC aggregates, but not MEF feeder cells, stain positive for both CDy1 (red) and Oct4 (green). Scale bar, 100  $\mu$ m. The images were kindly provided courtesy of Dr. Y-T Chang at the National University of Singapore, Republic of Singapore.

## How Are Researchers Using CDy1?

Recent publications highlight the advantages of using CDy1 for identification, enrichment and characterization of various stem cell types including embryonic, reprogrammed, neural and cancer stem cells. For example, CDy1 was shown to identify iPSCs post retroviral infection of OSKM reprogramming transcription factors 7 days prior to a GFP reporter system.<sup>4</sup> CDy1 has been used by researchers for various applications, such as to enrich for neural stem cells, as shown by work from the laboratory of Dr. Geoffrey W. Osborne in Queensland,<sup>2</sup> and to select for drug-resistant cancer stem cells, as demonstrated by Hawley *et al.* at George Washington University!<sup>1</sup>

For an up-to-date list of publications, information and data on the CDy1 Dye, visit [www.activemotif.com/cdy1](http://www.activemotif.com/cdy1).

## Recent CDy1 Publications:

1. Hawley *et al.* (2013) *Am J Hematol.* 88: 265-272.
2. Vukovic *et al.* (2013) *Stem Cells Dev.* Apr 27.
3. Vendrell *et al.* (2012) *Stem Cell Res.* 9: 185-191.
4. Kang *et al.* (2011) *Nature Protocols* 6: 1044-1052.
5. Im *et al.* (2010) *Angew. Chem. Int. Ed. Engl.* 49: 7497-7500.

## What's in the Box?

**Stem Cell CDy1 Dye** is supplied as a 50  $\mu$ l stock solution in DMSO.

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

Product	Format	Catalog No.
Stem Cell CDy1 Dye	50 $\mu$ l	14001

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