

ab308241 – EZCell™ CFDA SE Cell Tracer Kit

Flow cytometry based assay to determine cell proliferation using CFDA SE dye

For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: [abcam website](#)

Storage and Stability

Store the kit at -20°C, protected from light. Warm all the reagents to room temperature (RT) before use. Read the entire protocol before performing the assay.

Materials Supplied

Item	Quantity	Storage Condition
CFDA SE Assay Buffer	100 ml	4°C
CFDA SE (Avoid light)	5 vials	-20°C
DMSO (Avoid light)	1 ml	RT

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Tissue culture treated 6-well plate with clear bottom and lid
- Aluminum foil
- Sterile PBS
- Trypsin/0.25% EDTA
- Cell media
- Fetal bovine serum
- Flow cytometer

Reagent Preparation

CFDA SE Assay Buffer: Warm to 37°C before use. Store at 4°C.

CFDA SE: Add 179 µl of DMSO to one vial of CFDA SE to make 1 mM CFDA SE solution. Mix to dissolve completely and wrap the vial with aluminum foil to protect it from light. Store at -20°C for 2 months.

DMSO: Warm to RT to thaw completely before use

CFDA SE Cell Tracer Protocol:

Δ Note: *The assay was developed using 3T3 (adherent) and Jurkat (suspension) cells and can be modified for any other cell lines. The protocol below refers to a 6-well tissue culture plate. Adjust the volume accordingly for other plate formats. The assay volume is 1 ml. Optimize the CFDA SE solution for your cell type. We suggest testing several concentrations of the CFDA SE solution to find the best conditions for your experimental design. Cell density depends on the cell type, and it may be necessary to adjust the cell numbers for optimal cell density.*

Culture cells (3-5 x 10⁵ cells /well) in a 6-well plate format in 2 ml complete cell media containing 10% FBS and incubate for 24 hr.

Δ Note: *Optional: Incubate cells in complete cell media containing 10% FBS in the presence or absence of various test compounds for 1-24 hr. This step is only required for studying the potential effects of test compound(s) on cell division.*

Prepare 1 µM* Cell Tracer Staining solution by adding 40 µl of 1 mM CFDA SE solution to 40 ml PBS

Δ Note:

1. *For short term studies: We recommend using 0.05-5 µM Cell Tracer Staining solution.

2. *For long term studies: We recommend using 5-10 µM Cell Tracer Staining solution.

For Adherent Cells:

1. Remove complete cell media and wash the cells with 1 ml PBS.
2. Add 1000 µl of 1X Cell Tracer Staining solution and incubate at 37°C for 30 min.
3. Remove the supernatant and wash the cells with 1 ml PBS once.
4. Trypsinize and collect the cells in 2 ml complete cell media containing 10% FBS. Add 1 ml cells into one well of a 6-well plate and adjust the volume to 2 ml with complete cell media containing 10% FBS. Incubate the plate at 37°C, 5% CO₂ incubator for the experimentally required time for cell tracing studies. Transfer the remaining 1 ml cells into a 1.5 ml microcentrifuge tube.
5. Centrifuge the remaining cells at 500 x g for 5 min at 4°C. Remove the supernatant and resuspend cells in 500 µl CFDA SE Assay Buffer.
6. Record the fluorescence intensity using flow cytometer in FL-1 channel.
7. For sequential cell tracing studies: Repeat Step c-f.

For Suspension Cells:

1. Collect cells in a 1.5 ml microcentrifuge tube. Centrifuge cells at 500 x g for 5 min and remove the supernatant.
2. Add 1000 µl of 1 x Cell Tracing Staining solution, poke a hole in the lid by using 18 G 11/2 needle and incubate at 37°C for 30 min.
3. Centrifuge cells at 500 x g for 5 min at 4°C. and carefully remove the supernatant.
4. Wash cells once in 2 ml CFDA SE Assay Buffer, centrifuge at 500 x g for 5 min and carefully remove the supernatant.
5. Resuspend cells in 2 ml complete cell media containing 10% FBS. Add 1 ml cells into one well of a 6 well-plate and adjust the volume to 2 ml with complete cell media containing 10% FBS. Incubate the cells in 37°C, 5% CO₂ incubator for the experimentally required time for cell tracing studies. Transfer the remaining 1 ml cells into a 1.5 ml microcentrifuge tube.
6. Centrifuge the remaining cells at 500 x g for 5 min, remove the supernatant and resuspend cells in 1 ml CFDA SE Assay Buffer.
7. Record the fluorescence intensity using flow cytometer in FL-1 channel.
8. For sequential cell tracing studies: Repeat Step c-g.

Data Analysis:

Δ Note: *When analyzing the data, gate the cells of interest and exclude the cell debris and cell aggregates.*

Technical Support

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