AB269446 – Cell Tracking Red Dye Kit - Longer cell staining, DMSO-free

Cell Tracking Red Dye Kit ab269446 stains cell membranes in live cells and is ideal for use in cell tracking and cell labelling. It uses SomaServe's PolyNaut® dye-loading technology to enable long-term live cell staining and is used without DMSO in order to minimise cell toxicity.

For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/AB269446

Assay Procedure

1. Culture adherent cells on a surface that is compatible with available imaging systems (e.g. coverslip, chamber slide, clear-bottom optical multi-well plates, etc. Suspension cells should be prepared similarly, with centrifugation (200g, 5 minutes) before and after each washing step.

2. Determine the optimal cell density / number for each assay experimentally; 104 - 105 cells per well is an initial recommendation if using a 96-well plate.

3. To prepare the labelling solution, dilute ab269446 1:20 with fresh cell culture medium; adding 1 part of ab269446 to 19 parts of medium.

4. Serum in the medium will not interfere with the labelling. We recommend 100 µl of labelling solution per well if using a 96-well plate. For live cell imaging, use phenol red-free cell culture medium.

5. Add labelling solution to the well.

6. Instead of preparing a labelling solution, ab269446 may be added directly to the media (e.g. 5 µL of ab269446 added to 100 µL of cell culture media).

7. Incubate at 37 °C, 5% CO2, for 30 minutes to 96 h.

8. Labelling generally increases over the first 10 h as uptake of the polysomes and release of the dye takes time. The optimal incubation time depends on cell type, e.g. tumour cells usually uptake faster than primary cells. As a minimum, we recommend 30 min incubation with labelling solution. If cells are not sufficiently labelled after this time, we recommend increasing the labelling time to 2h, 10 h, etc., up to 96 h.

9. Labelled cells can be observed by microscopy (Ex max 554 nm, Em max 575 nm) while they are in labelling solution. Alternatively, the labelling solution can be removed at any given time point by 3x washing with fresh culture media. After removing the labelling solution, depending on cell type, cells will remain labelled for up to 6 days or more.

10. Optional: If DNA counterstaining with Hoechst 33342 (ab228551) is required, we recommend diluting ab228551 at 1:10,000 in cell culture media and incubating for 15 min. Wash cells two times with pre-warmed fresh cell culture medium.

11. If required, cells labelled with ab269446 can be fixed with 4% PFA (although this tends to shrink the cells and make it harder to achieve high quality images). Note that MeOH fixation is not compatible with this product.

For technical support contact information, visit: www.abcam.com/contactus

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