

**CellPaint™ TSP membrane stain**

 Catalog number: 22700  
 Unit size: 500 Tests

Component	Storage	Amount
Cellpaint™ TSP membrane stain	Freeze (< -15 °C), Minimize light exposure	500 Tests

**OVERVIEW**

TSP is a styrylpyridine-based fluorescent membrane probe suitable for imaging plasma membranes in living cells and tissues. It was reported by Guo et al in 2016 (Analyst, 2016, 141, 3228). The probe is a molecular rotor that has fluorescence sharply enhanced in viscous media. Its fluorescence is also microenvironment-sensitive, enables the turn-on imaging of plasma membranes with a high signal-to-noise ratio. Guo et al has demonstrated that TSP has high photostability, low cytotoxicity and excellent biocompatibility. It can also be used in 2 photon imaging.

**AT A GLANCE**

1. Prepare cells in growth medium
2. Incubate cells with Cellpaint™ TSP membrane stain
3. Analyze under fluorescence microscope with Cy3/TRITC filter set

**Chemical and Physical Properties**

Molecular Weight: 1016.27

Solvent: dimethyl sulfoxide (DMSO)

Spectral Properties: Excitation = 490 nm; Emission = 600 nm

**KEY PARAMETERS**
**Fluorescence microscope**

Excitation	Cy3/TRITC filter set
Emission	Cy3/TRITC filter set
Recommended plate	Black wall/clear bottom

**CELL PREPARATION**

For guidelines on cell sample preparation, please visit <https://www.aatbio.com/resources/guides/cell-sample-preparation.html>

**PREPARATION OF STOCK SOLUTIONS**

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

**Cellpaint™ TSP membrane stain stock solution (500X)**

1. Thaw the vial of CellPaint™ TSP membrane stain at room temperature before use.
2. Add 100 µL of DMSO into the vial of Cellpaint™ TSP membrane stain to make 500X stock solution.

**Note** Unused Cellpaint™ TSP membrane stain stock solution can be aliquoted and stored at ≤ -20 °C for a couple of months if the tubes are sealed tightly. Protect from light and avoid repeated freeze-thaw cycles.

**PREPARATION OF WORKING SOLUTION**
**Cellpaint™ TSP membrane stain working solution (10X)**

1. Add 20 µL of 500X stock solution into 1 mL of cell culture medium, and mix well.

**Note** We recommend making the working solution fresh before use.

**Note** 20 µL of 500X Cellpaint™ TSP membrane stain stock solution is enough for one 96-well plate.

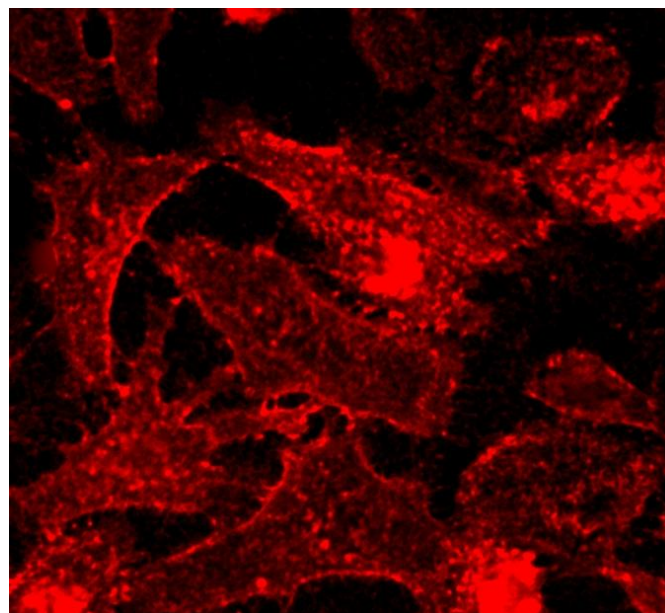
**Note** We recommend using a cell culture medium to make a working solution for better resolution images.

**SAMPLE EXPERIMENTAL PROTOCOL**
**Stain Cells**

1. Add 10 µL/well (96-well plate/100 µL volume) or 5 µL/well (384-well plate-50 µL volume) of 10X Cellpaint™ TSP membrane stain working solution in the cell plate. Incubate the cells at 37 °C for 30-60 minutes, protected from light.

**Note** The optimal concentration of the cell membrane probe varies depending on the specific application. The staining conditions may be modified according to the particular cell type and the permeability of the cells or tissues to the probe.

2. Remove working solution in each well. Wash cells with physiological buffer (such as HHBS, DPBS or buffer of your choice) for three times and replace with HHBS.
3. Observe the fluorescence signal in cells using fluorescence microscope with a Cy3/TRITC filter set.

**EXAMPLE DATA ANALYSIS AND FIGURES**


**Figure 1.** HeLa cells were labeled with CellPaint™ TSP membrane stain (Cat No. 22700). Labeled cells were imaged on the Keyence BZ-X710 All-In-One Fluorescence Microscope equipped with a Cy3/TRITC filter set.

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