Transmembrane Protein Extraction Reagent

Cat No. TmPER-50 (30 ml); TmPER-100 (50 ml); TmPER-200 (100 ml)
Storage: 4°C

Precautions:
Contains irritants. Use gloves and eye protection. Avoid ingestion, eye and skin contact.

Required items that are not provided:
PBS for cell washes, protease inhibitors, cell scraper, refrigerated microcentrifuge.

Preparation:
The reagent is provided as a 1X solution. Aliquots of Transmembrane Protein Extraction Reagent should be cooled in a tube in ice prior to use. Add protease inhibitors immediately prior to use.

Overview

Large molecular weight (120 kD and above) transmembrane proteins pose challenges to membrane extraction required for Western blot analysis. A standard cell lysis buffer, including RIPA buffer, is often insufficient to extract and solubilize proteins in this category. Examples of proteins meeting these criteria include ABC transporters, such as CFTR, ATPase-Pumps, including SERCA, calcium channels, ion exchangers and some GPCR receptors. The FIVEphoton Biochemicals Transmembrane Protein Extraction Reagent contains a proprietary formulation that substantially increases transmembrane protein solubilization and extraction.

In the first step, the Transmembrane Protein Extraction Reagent is dispensed on cells on a culture dish (after removal of media and washing with PBS). Cells are then gently scraped from the culture dish and added to a separate tube as a semi-soluble suspension. Vortexing is employed to promote dissolution of the membrane and protein solubilization and extraction. The resulting cell suspension is kept on ice with occasional vortexing. After a 30 minute to 2 hour period for membrane dissolution, a centrifugation step is used to remove insoluble cellular debris. The supernatant fraction, which contains extracted transmembrane protein, is collected and suspended in Laemmli Sample Buffer, heated between 37-60°C (without boiling, to prevent insolubility of large transmembrane proteins), and resolved in SDS-PAGE gels.

The Western blot image displayed in the product description on the fivephoton.com website is an example of a high molecular weight multi-pass ATP-dependent transporter protein that does not adequately extract in RIPA buffer and requires the Transmembrane Protein Extraction Reagent formulation.

Protocol

A. Cell Culture. Grow cells in cell culture dishes until 80% to 90% confluency. A semi-confluent cell culture population yields larger amounts of extracted protein. To limit endocytosis prior to extraction, place the cell culture dish with media on a bed of ice for approximately 15 minutes prior to removal of the cell culture media. Another method to limit endocytosis is to replace the cell culture media with ice cold PBS containing 1.0 mM $MgCl_2$ and 0.1 mM $CaCl_2$ for 15 minutes prior to cell lysing.

B. Preparation of Transmembrane Protein Extraction Reagent. Aliquot approximately 300 µl of 1X Transmembrane Protein Extraction Reagent for each 10 cm cell culture dish. (Calculate corresponding volumes for other differently sized culture dishes depending on the surface area of the dish). Add a general protease inhibitor cocktail as well as a serine protease inhibitor (e.g. PMSF) to ice-cold Transmembrane Protein Extraction Reagent immediately prior to use.

C. Dissolution of Cells. A recommended method to remove cells from the culture dish and how to apply the Transmembrane Protein Extraction Reagent is as follows:

Wash cells with PBS: Remove media using a Pasteur pipette linked to a vacuum line. Remove as much media as possible. Gently add 3 ml of ice-cold PBS, rotate the cell culture dish gently, and then remove the PBS. Repeat the PBS wash procedure 2X. The PBS washes remove serum and other proteins in the media that may add spurious bands on subsequent Western Blots. If cells are incubated in ice-cold PBS with calcium chloride and magnesium

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chloride, simply remove the PBS solution by suction prior to the addition of Transmembrane Protein Extraction Reagent.

Solubilize cell membrane: Dispense 300 μl of ice cold Transmembrane Protein Extraction Reagent onto each 10 cm plate. Rotate the culture dish to evenly disperse the Transmembrane Protein Extraction Reagent. Use a cell scraper to detach cells, and to collect cells into a corner of the dish. Siphon cells suspended in Transmembrane Protein Extraction Reagent with a plastic pipette applicator and dispense the cell solution into a 1.5 ml tube (or larger tube if needed). Vortex the tube rapidly at full speed for approximately 15 seconds, and then place the tube on ice for 30 minutes to 2 hours. Vortex the tube for 30 seconds approximately every 15 minutes during this period.

D. Centrifugation to isolate solubilized transmembrane proteins. Separate the supernatant, which contains the soluble transmembrane protein fraction, from insoluble debris as follows:

After the 30 minute to 2 hour extraction period in ice, centrifuge the 1.5 ml tube in a microcentrifuge ($4^{o}C$) at top speed for 15 minutes. The supernatant fraction contains the extracted transmembrane proteins (as well as cytosolic proteins).

Isolate supernatant from pellet, and dispense the supernatant into a separate tube. At this point, you may opt to perform protein assays to estimate protein concentration.

Aliquoting and storage of the supernatant fraction at $-20^{o}C$ or $-80^{o}C$ for later use (without the addition of Laemmli Sample Buffer) is a recommended procedure. For long term storage, store aliquots at $-80^{o}C$.

E. Preparation of Sample for Western Blot Analysis. Dilute an aliquot of supernatant into Laemmli Sample Buffer with added 5% β-mercaptoethanol. Vortex rapidly for 30 seconds, and then either heat at $37^{o}C$ for 30 minutes or $60^{o}C$ for 10 minutes. Load and resolve samples in SDS-PAGE gels on the same day as extraction or store Laemmli Sample Buffer solution with extracted proteins at $4^{o}C$ prior to loading of gels. The best results are often obtained when the sample is resolved in SDS-PAGE gels on the same day as extraction.

Samples suspended in Laemmli Sample Buffer can be stored for approximately one week at $4^{o}C$. If performing Western Blot transfer, we recommend using 75% of the methanol typically used in the transfer buffer, as well as adding 0.1% SDS in the transfer buffer. Perform the transfer approximately 25% longer to remove large proteins from gels.

Troubleshooting

1. Cells do not appear to lyze with the recommended Transmembrane Protein Extraction Reagent volume. Resolution: After vortexing the cell suspension in Transmembrane Extraction Reagent, you should observe semi-suspended threaded materials which correspond to the DNA removed from cells. If DNA is not observed, gradually add small aliquots of Transmembrane Protein Extraction Reagent to the cell suspension and vortex. Continue to add Transmembrane Protein Extraction Reagent until DNA threads are observed. The appearance of DNA threads indicates that the cell membrane was lysed.

2. Protein bands are not resolved clearly in Western blots. Resolution: The transmembrane protein of interest may have become insoluble during the $60^{o}C$ heating process in Laemmli Sample Buffer. Resuspend a new aliquot of cellular extract in Laemmli Sample Buffer and heat at $37^{o}C$ for 30 minutes. Vortex two to three times during the heating period. Now resolve the sample in SDS-PAGE gels and Western blots.

3. The transmembrane protein target was clearly resolvable in Western blots when the extract-Laemmli Sample Buffer solution was first resolved in gels, but is no longer present after extended storage at $4^{o}C$. Resolution: Defrost a new aliquot of sample, and prepare in Laemmli Sample Buffer immediately prior to resolution in SDS-PAGE gels.

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