INSTRUCTIONS

Chrometra Tubulin ExM

Polymerizable Taxane derivatives

Product Numbers	Description
ER-15 range	Reagent for staining of tubulin structures in microscopy. **Contents:** Reactive taxane derivative (100 or 300 reactions) **Storage** Upon receipt store product at -20°C.

Introduction

Chrometra Tubulin ExM for staining of tubulin networks in polymeric gels and general fluorescence microscopy.

The Chrometra Tubulin ExM kit is used to introduce reactive labels onto microtubules. By incorporating a polymerizable moiety into the probe, the conjugate becomes covalently anchored to the swellable gel during polymerization, preserving the tubulin signal through digestion and isotropic expansion. The reagent is available containing either a reactive azide or a biotin.

Kit Contents

- 1 or 3 vials of Tubulin ExM grafting reagent (100 or 300 reactions, 30 reactions for samples).
- 1 or 3 vials of fluorescent Post Stain reagent
- Optional: 20 mM N-Ethyl Maleimide (NEM) (see recipe below).

Reagents not included but which may be required:

- Glutaraldehyde
- $NaBH_4$ as a 0.15% (w/v) solution in PBS
- PBS Buffer
- PBS Buffer containing 0.1% Tween-20
- PBS + 1% BSA
- PEM solution (80 mM PIPES pH 6.8, 5 mM Ethylene glycol tetraacetic acid (EGTA), 1 mM MgCl2 and 0.3% Triton X-100 (see recipe below).

Reagent preparation

The grafting reagent is supplied dry. Reconstitute it in DMSO or methanol by adding 100 µL of solvent to the tube and incubating at 37 °C with gentle shaking for 5 min. Immediately before staining, dilute 1 µL of this stock into 50 µL of an appropriate buffer (e.g., PBS; not provided). Reconstitute the Post stain reagent in DMSO by adding 100 µL of solvent to the tube and incubating at 37 °C with gentle shaking for 5 min. 1 µL of this stock corresponds to one unit. Store both reagents frozen.

Procedure for tubulin grafting (default)

1. Incubate the sample in 3 µM docetaxel probe for 1 h at 37 °C.
2. Fix in 0.5% GA in PEM + 0.3% Triton X-100 for 10 min.
3. Rinse once with PBS.
4. Quench residual GA with 0.15% $NaBH_4$ in PBS for 7 min at RT.
5. Wash the cells with PBS buffer (3 times, for 5 minutes)
6. Optional: Incubate in 20 mM NEM (PBS) for 30 min to block free thiols
7. Wash the cells with PBS buffer (3 times, for 5 minutes)
8. Proceed to staining through Click Labeling (Procedure A, B) or direct streptavidin staining (Procedure C)

Labeling Procedure A: Click Labeling (Biotin/Streptavidin example, for use with Tubulin ExM Biotin)

1. Incubate with 10 µM of in PBS + 1% BSA.
2. Wash with PBS + 0.1% Tween-20. (3 times, for 5 minutes)
3. Incubate with fluorescent streptavidin (15 µg/mL) in PBS + 1% BSA for 1 h.
4. Wash the cells with PBS buffer (3 times, for 5 minutes)
5. Optional: Nuclear counterstain: Incubate with 1 µg/mL DAPI for 1 min.
6. Wash the cells with PBS buffer (3 times, for 5 minutes)
7. Proceed to gelation or imaging

Labeling Procedure B: Click Labeling (Dye example, for use with Tubulin ExM Post)

1. After the default tubulin grafting, incubate with 1 unit of Post Stain reagent dye in PBS + 1% BSA.
2. Wash the cells with PBS buffer + 0.1% Tween-20 (3 times, for 5 minutes)
3. Proceed to gelation or imaging

Labeling procedure C: Direct streptavidin staining (for use with Tubulin ExM Biotin)

1. Incubate with fluorescent streptavidin (15 µg/mL) in PBS + 1% BSA for 1 h.
2. Wash the cells with PBS buffer (3 times, for 5 minutes)
3. Proceed to gelation or imaging

Example Calculations for stock solutions:

0.15% (w/v) $NaBH_4$ in PBS:

Weigh 1.5 mg $NaBH_4$ and bring up to 1.00 mL with PBS (prepare fresh, cold if possible).

20 mM N-ethylmaleimide (NEM) in PBS:

Bring the provided tube of NEM up to 1.00 mL with PBS.

PEM solution (10 mL recipe):

1. Weigh the following reagents:
    a. PIPES (80 mM; 0.8 mmol): PIPES free acid (MW 302.37): 242 mg, or PIPES disodium (MW 366.35): 293.1 mg.
    b. EGTA (5 mM; 50 µmol): EGTA free acid (MW 380.35): 19.0 mg or EGTA disodium (MW 436.31): 21.8 mg.
    c. $MgCl_2$ (1 mM; 10 µmol): Anhydrous (MW 95.21): 0.952 mg, or Hexahydrate (MW 203.30): 2.03 mg. (Practical alternative: add 10 µL of a 1 M $MgCl_2$ stock.)
    d. Triton X-100 (0.3% v/v): 30 µL

2. Prepare the solution:
    a. Dissolve PIPES and EGTA in ~8 mL ultrapure H_2O.
    b. Adjust to pH 6.8 with NaOH.
    c. Add $MgCl_2$ (weighed or from 1M stock).
    d. Add 30 µL Triton X-100, mix gently.
    e. Bring volume to 10 mL with H_2O
    f. Optional: filter-sterilize; store cold.

Example gelation procedure (4x Expansion)

1. Incubate samples in 0.1 mg/ml crosslinker (e.g.; Acrylink NHS (0.35 mM), Acrylink TFP (0.3 mM), AcX (0.35 mM)) in PBS for 90 min at RT.
2. Wash the cells with PBS buffer (3 times, for 5 minutes)
3. Prepare gelation mix (monomer + initiators): 2 M NaCl, 8.625% (w/w) sodium acrylate, 2.5% (w/w) acrylamide, 0.15% (w/w) N,N'-methylenebisacrylamide, 0.01% 4-hydroxy-TEMPO.
4. Immediately before use, add 0.15% (w/w) TEMED and 0.15% (w/w) APS to the required aliquot. Mix gently.
5. Quickly rinse each sample with 50 µL of the freshly initiated monomer solution to pre-infuse.
6. Assemble the sample in a gelation chamber.

7. Cover the sample in gelation solution
8. Polymerize in a humidified incubator at 37 °C for 90 min (avoid evaporation).
9. Digest by incubating the gels with Proteinase K, 8 U/mL in digestion buffer (50 mM Tris, pH 8.0; 1 mM EDTA; 0.5% Triton X-100; 0.8 M guanidine HCl) at 37 °C for 3 h.
10. Optional nuclear counterstain: Stain with DAPI, 1 µg/mL in PBS for 1 min; Wash the cells with PBS buffer (3 times)
11. Transfer gels to deionized H_2O for 10 min.
12. Replace with fresh H_2O and repeat 4× (or until dimension change plateaus) to reach full 4× expansion.

Storage and Safety

The Tubulin ExM reagent can be stored at 4°C for 3 months. For longer storage the conjugate can be stored at -20°C. The conjugate should always be stored in the dark.

Tips and troubleshooting

- To avoid photobleaching of dilute dyes, minimize the exposure of fluorescently labeled specimens to light.
- Dye concentration: When following the standard guidelines, one unit of dye reagent in a staining buffer solution of 100 µl will result in a 5 µM concentration. Dye working concentrations can be reduced to accommodate for larger volumes of staining solution. In such case, extend incubation to 2 hours at 37°C.
- The addition of 0.1 mg/ml general linker (e.g.; Acrylink NHS (0.35 mM), Acrylink TFP (0.3 mM), AcX (0.35 mM)) has been reported to increase S/N in several sample types.
- Avoid contact of any dye containing reagent with the $NaBH_4$ solution, as many dyes will lose fluorescence.
- The NEM thiol blocking step is optional, and can be used when background staining is observed.