PROTOCOL

Western blot Procedure for MAb to PMCA ATPase (5F10) (Prod. No. ALX-804-049):

1. Transfer proteins to nitrocellulose filters using a transfer buffer which lacks methanol, but is high in glycine (0.7M glycine, 25mM TRIS free base).
2. Incubate filters for 1 hour in 10mg/ml BSA in PBS, pH 7.4, to block non-specific binding.
3. Wash with PBS.
4. Incubate filters for 1 hour at room temperature with MAb to PMCA ATPase (5F10) (Prod. No. ALX-804-049) diluted 1:1'000 in PBS.
5. Wash with PBS 3 x for 10 minutes.
6. Incubate filters for 30 minutes at room temperature with AffiniClear™ HRP-conjugated donkey antibody to mouse.
7. Wash with PBS 3 x for 10 minutes.
8. React filters with diaminobenzidine tetrahydrochloride in 0.1% hydrogen peroxide to effect. Stop reaction (usually after 0.5-10 minutes) by rinsing with distilled water.

Note: Use 7% SDS-PAGE. 5ng PMCA is readily detected using a 1: 1'000 dilution of MAb to PMCA ATPase (5F10) (Prod. No. ALX-804-049).

Immunohistochemical Detection of PMCA in formalin fixed paraffin embedded tissues using MAb to PMCA ATPase (5F10) (Prod. No. ALX-804-049):

1. Mount 4 micron sections on resin-coated slides. Deparaffinize in xylene and rehydrate in graduated alcohols to distilled water.
2. Block endogenous peroxidase by incubating in 0.5% hydrogen peroxide for 10 minutes.
3. Wash in 0.01M PBS, pH 7.2, 3 x for 5 minutes.
4. Incubate in 10% normal goat serum for 10 minutes.
5. Tap off goat serum and incubate with MAb to PMCA ATPase (5F10) (Prod. No. ALX-804-049) diluted 1:500 in PBS 60 minutes at room temperature (RT) in humid chamber.
6. Wash with PBS 3 x for 5 minutes.
7. Incubate with biotinylated goat secondary antibody to mouse for 10 minutes at RT in humid chamber.
8. Wash with PBS 3 x for 5 minutes.
9. Incubate with streptavidin/peroxidase conjugate for 5 minutes at RT in humid chamber.
10. React with chromagen substrate 0.05% DAB 5-15 minutes at RT in humid chamber. Stop reaction with distilled water.
11. Counterstain with hematoxylin for 3 minutes at RT in humid chamber.
12. Dehydrate in sequential baths of xylene and mount with permount.

The procedures listed above are intended only as a guide. Various assay conditions require that the investigator determine the optimal working concentrations. The results may vary depending on experimental conditions and technique. No warranty or guarantee of performance of above procedure is made or implied. Use good laboratory practices and handle all materials with care.

These products and procedures are for in vitro experimental use only and are not intended for use in humans or clinical diagnosis.