

Western Blot Protocol using NF-ATc1, mAb (7A6) (Prod. No. ALX-804-022):

Preparing Gel and Samples:

- Nuclear extracts of induced cells may be ammonium sulfate precipitated prior to loading on gel.
- Separate proteins for detection on an 8% gel.

Blocking:

- Block nitrocellulose filter in TBST* containing 5% non-fat dried milk for 1 – 2 hours at room temperature with gentle agitation.

Antibody Incubation:

- Rinse filters with TBST
- Incubate for 1 hour with gentle agitation at room temperature with primary antibody (Prod. No. ALX-804-022) diluted in TBST containing 5% non-fat dried milk.
- Pour off antibody solution and wash 3 x 15 minutes with TBST at room temperature with gentle agitation.
- Incubate filters with AffiniClear™ HRP-conjugated donkey anti-mouse antibody diluted in TBST containing 5% non-fat dried milk for 1 hour at room temperature.
- Pour off antibody solution and wash 3 x 15 minutes with TBST at room temperature with gentle agitation.

Detection:

Detect antibody reaction by ECL according to manufacturers directions.

*Reagents: TBST, pH 8.0:
 10mM TRIS-HCL
 500mM NaCl
 0.1% Tween 20

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.