

# Protocol for Monocarboxylate transporter1 Antibody (Cat. No. 356 003)

## Western Blot (WB)

## ECL Detection

In standard Western blot (WB) approaches, protein samples are separated according to their molecular weight with denaturing SDS-PAGE (polyacrylamide gel electrophoresis), transferred to a membrane and analyzed by immuno-detection with antibodies. Enhanced chemiluminescent (ECL) detection systems and substrates have different sensitivities and have a narrow linear detection range that can be used for protein quantification. In general, the experiment has to be carefully optimized for reliable results.

## Materials and reagents

- **Membrane staining solution (optional):** suitable staining solution (e.g. Ponceau S)
- **TBST - skimmed milk:** (20 mM Tris, 150 mM NaCl, 5% (w/v) skimmed milk powder, 0.1% Tween 20), pH 7.4
- **Washing solution A:** (20 mM Tris, 150 mM NaCl, 0.1% Tween 20), pH 7.4
- **Washing solution B:** (20 mM Tris, 150 mM NaCl), pH 7.4
- **Primary antibody:** Monocarboxylate transporter1 antibody (cat. no. 356 003)
- **Secondary antibody:** Horseradish conjugated rabbit specific secondary antibody.
- **Substrate solution:** Western Lightning® Plus-ECL PerkinElmer, Inc. or comparable product

## Procedure

1. Prepare samples (10-20 µg / lane for a 10 lane mini-gel) in reducing sample buffer according to the manufacturer recommendation. If purified proteins or peptides are loaded use 1ng-1µg / lane for a 10 lane mini gel.
2. Incubate samples for 5 min at 95°C.
3. Separate the protein samples to be examined next to a molecular weight standard using SDS-PAGE and transfer to a nitrocellulose membrane by electro-blotting. Follow the manufacturer instructions for your SDS-PAGE and blotting device.
4. *Optional: Stain the membrane with membrane staining solution to check protein transfer.*
5. Incubate in **TBST - skimmed milk** for 30 min on an orbital shaker at RT.
6. Wash 3-4 times with **washing solution A** for 10 min each.
7. Incubate with fresh **TBST - skimmed milk** containing the **secondary antibody** diluted according to the manufacturer recommendations for at least 1 h on an orbital shaker.
8. Wash 3 times with **washing solution A** for 10 min each.

9. Replace **washing solution A** with **washing solution B** and equilibrate for 5 min.
10. Replace with fresh **substrate solution** and develop (X-ray film or ECL-reader). Exposure time can be shortened or extended, if signals are extremely strong or weak, resp.

## Remarks

For pre-adsorption specificity tests, please refer to our general [pre-adsorption protocol](#).

A very weak signal may be caused by the primary and/or secondary antibody concentration being too high. The ECL substrate solution has a limited capacity, and high amounts of local peroxidase can use up all the substrate within seconds before the picture is taken in your ECL reader.

Please try a lower concentration of primary and secondary antibodies in this case.

*Note: This protocol has been validated in the SYSY Antibodies laboratories to ensure consistent and reliable staining results.*

*However, for achieving the best specific signal with minimal background, the optimal antibody concentration, incubation temperature, and incubation duration should be optimized for each experiment.*