

Product No. 05999

# Blocking One-P

Blocking One-P is a blocking buffer for ELISA, Immunoblotting and immunohistochemistry. Especially, Blocking One-P is designed for applications with phospho-specific antibodies.

## Features

- Does not contain phosphorylated proteins.
- Phosphatase free.
- The preservative in the product does not affect the enzyme activity of horseradish peroxidase (HRP).
- Store the product in refrigerator after opening.

## Preparation of the blocking solution

### Blocking microplate for ELISA

Mix 32 ml deionized water and 8 ml Blocking One-P in a clean 50 ml beaker.

(Ratio: deionized water: Blocking One-P = 4:1)

For phospho-specific antibodies, we recommend adding phosphatase inhibitor (ex. Phosphatase Inhibitor Cocktail, Product No. 07574) to inhibit dephosphorylation of the sample.

### Dilution buffer of antibodies (For one microplate)

Mix 19 ml TBS containing 0.1 % Tween<sup>®</sup>20 ( tTBS ) with 1 ml Blocking One-P in a clean 20 ml beaker.

(Ratio: tTBS : Blocking One-P = 19 : 1)

For phospho-specific antibodies, we recommend adding phosphatase inhibitor (ex. Phosphatase Inhibitor Cocktail) to inhibit dephosphorylation of the sample.

### Blocking for immunoblotting membrane

Use Blocking One-P as it is.

## Protocol

### For blocking in 96-well microplate

Apply 350µl prepared blocking solution with a micropipette into each well of the microplate and let it stand for 45 minutes at room temperature. Remove the blocking solution from the wells by turning the microplate upside-down. Apply 350µl tTBS into the wells of the microplate to rinse out the blocking solution. Repeat 3 times. The plate is ready for antigen-antibody reaction. For phospho-specific antibodies, we recommend adding phosphatase inhibitor (ex. Phosphatase Inhibitor Cocktail) to inhibit dephosphorylation of the sample.

### For diluting of enzyme labeled antibody

Add the enzyme labeled antibody of predetermined quantity into blocking solution diluted 20 times. Shake carefully not to generate foam, and use immediately. Activity of the enzyme labeled antibody after dilution or the sensitivity of ELISA test may decrease over time. For phosphor-specific antibodies, we recommend adding phosphatase inhibitor (ex. Phosphatase Inhibitor Cocktail) to inhibit dephosphorylation of the sample.

### For blocking of immunoblotting membrane

For a membrane (approx. 10 cm x 10 cm), pour 50 ml Blocking One-P into a clean plastic tray. Immerse the membrane into the solution and shake for approx. 20 minutes.

## Caution

- Blocking One-P contains purified protein ingredient. In rare cases cross-reaction with the antibody may occur. Use Blocking One-P after confirmation of no crossreaction.
- The product cannot be used as buffer solution for alkaline phosphatase labeled antibodies. If using alkaline phosphatase labeled antibody, wash the membrane thoroughly and carefully after blocking with Blocking One-P.
- For phospho-specific antibodies, we recommend adding phosphatase inhibitor (ex. Phosphatase Inhibitor Cocktail) to inhibit dephosphorylation of the sample.
- If the blocking effect is too strong, dilute the product with TBS or shorten blocking time.

---

**Storage**

Refrigerator

---

**Expiration**

Expiration date is stated on the product label.

---

**Packing**

200 ml (Product No.05999-84)