

Anti-Phosphotyrosine Western Blotting Kit
(HRP-conjugated 4G10)
Catalog # 17-123
Lot # 19176

Kit Components

HRP-conjugated 4G10 Anti-Phosphotyrosine, Catalog # 16-105, Lot # 18633, see page two for more information. One vial containing **100mg** of IgG_{2bk}-HRP conjugate in **65ml** PBS containing 0.01% merthiolate and 10% glycerol. Sufficient for **10 immunoblots** if used at the optimum concentration of 1µg/ml in 10ml blocking buffer. Liquid.

Phosphorylated A431 Protein Preparation. (Positive Control), Catalog # 12-110, Lot # 18458, see page two for more information. Two vials, each containing **500mg** in **250ml**, for a total of 1mg, with a final concentration of 2mg/ml protein.

Blocking Buffer, Catalog # 17-105b, Lot # 18912. One vial containing **20g**/vial. Non-fat skim milk.

Kit Description

Quantity: 10 blots per kit.

Quality Control: The reagents in this kit have been carefully matched with special attention directed to the sensitivity of detection and the 'strength' of the signal produced.

Storage and Stability: Stable for 6 months at 2-8°C from date of shipment. **Note:** Store Catalog # 12-110, Phosphorylated A431 Protein Preparation at -20°C for optimal performance. Do not use sodium azide with Catalog # 16-105, as it is an inhibitor of horseradish peroxidase.

Use: Read the enclosed protocol before use.

FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR IN ANIMALS

Technical Information for Kit Components

HRP-conjugated Anti-Phosphotyrosine (monoclonal IgG_{2bk})

Antibody Class: IgG_{2bk} mouse monoclonal antibody produced *in vitro* by mouse-mouse hybridoma 4G10 (FOX-NY [NS-1 derivative] myeloma x spleen cells). Purified by Protein A-Sepharose chromatography and cross-linked to horseradish peroxidase.

Note: Centrifuge the original vial prior to removing the cap for maximum recovery of the product.

Immunogen: Phosphotyramine-KLH.

Physical Form: Liquid.

References:

Cohen, B., *et al.*, Proc. Natl. Acad. Sci. USA **87**: 4458-4462, 1990.
Druker, B.J., *et al.*, New Eng. J. Med. **321**: 1383-1391, 1989.
Kanakura, Y., *et al.*, J. Biol. Chem. **266**: 490-495, 1991.

Phosphorylated A431 Protein Prep. (in RIPA Buffer)

Product Description: Cellular protein preparation from A431 cells containing phosphorylated EGF receptor. A431 cells were cultured for 20 minutes in the presence of 50ng/ml EGF. Cells were lysed in modified RIPA buffer (50mM Tris-HCl, pH 7.4, 1% NP40, 0.25% sodium deoxycholate, 150mM NaCl, 1mM EGTA, 1mM PMSF, 1µg/ml aprotinin, 1µg/ml leupeptin, 1µg/ml pepstatin, 1mM Na₃VO₄, 1mM NaF).

Physical Form: Frozen solution.

Storage: For maximum recovery of the product centrifuge the original vial after thawing and prior to removing the cap. Aliquot to avoid repeated freezing and thawing.

Western Immunoblot Analysis Protocol

Reagent Preparation:

Blocking Buffer: Dissolve 0.9g of "Blocking Reagent," Catalog. # 17-105b in 30ml of phosphate buffered saline (PBS), pH 7.4. Check pH.

Primary Antibody Solution: Add 10µg of HRP-conjugated 4G10 Anti-Phosphotyrosine Antibody, Catalog # 16-105, to 10ml of **FRESHLY** prepared "Blocking Buffer". Mix well and store at 2-8°C. Some tyrosine phosphorylated proteins may require more antibody to be detected. **NOTE:** This solution can be reused ONCE within one week.

Procedure:

Prior to Immunodetection:

1. Prepare the samples and "Positive Control" (Catalog # 12-110) for electrophoresis and blotting. Load the SDS-PAGE gel with the samples to be tested and 10µl of the Positive Control.

Note: A positive control should be included with every analysis.

2. Perform electrophoresis and blot to nitrocellulose filter paper.
3. Wash the nitrocellulose filter two times with fresh changes of PBS (phosphate buffered saline) or distilled water.

Immunodetection:

1. Block the blotted nitrocellulose filter by immersing in 10ml of **freshly** prepared "Blocking Buffer" for 30 minutes at room temperature.
2. Incubate the nitrocellulose filter in the "Primary Antibody Solution" overnight at 2-8°C. The solution can be saved for one additional use.
3. Wash the nitrocellulose filter two times with fresh changes of PBS or distilled water.
4. Wash the nitrocellulose filter in PBS-0.05% Tween 20 for 3-5 minutes with constant rocking.
5. Rinse the nitrocellulose filter four times with fresh changes of PBS or distilled water.
6. **The nitrocellulose filter membrane can be developed by one of the following two methods.**
 - A. Incubate the nitrocellulose in developing reagent [1ml chloronaphthol solution, 30mg/ml in methanol), 10ml methanol, 39ml TBS, and 30µl of freshly prepared 30% hydrogen peroxide (H₂O₂)] for 5-30 minutes. Replace developing reagent as heavy precipitate forms. Stop development by washing membrane with distilled water (3 changes in 30 minutes). Photocopy or photograph to preserve a permanent record.
 - B. Follow with standard enhanced chemiluminescence (ECL) detection reagents. ECL detection is **highly** recommended (a 1 minute exposure was used when testing this lot).