

---

## Certificate of Analysis

### Catch and Release<sup>®</sup> Phosphotyrosine, clone 4G10<sup>®</sup> Immunoprecipitation Kit Catalog # 17-502 Lot # 33357

#### Kit Components

**Anti-Phosphotyrosine, recombinant clone 4G10<sup>®</sup>**, Catalog # 05-777B. One vial containing **250µg** of protein G purified, recombinant 4G10<sup>®</sup> mouse IgG<sub>2bκ</sub> in 267µl of PBS, pH 7.5. Liquid at 4°C. Store at 4°C.

**Catch and Release<sup>®</sup> Wash Buffer, 10X**, Catalog # 20-210. One vial containing **15ml** of 10X buffer, pH 7.4 containing the following detergents: 10% NP-40, 2.5% deoxycholic acid and 150mM imidazole. Store at 4°C. **Note:** If crystallization occurs when buffer is stored at 4°C, warm to room temperature and vortex briefly before use.

**Catch and Release<sup>®</sup> Non-denaturing Elution Buffer, 4X**, Catalog # 20-209. One vial containing **10ml** of 4X PBS-based IP Elution Buffer. Store at 4°C.

**Catch and Release<sup>®</sup> Denaturing Elution Buffer, 1X**, Catalog # 20-284. One vial containing **4ml** of 1X Tris-based IP Elution Buffer. Add β-mercaptoethanol (bME) to a final concentration of 5% v/v immediately before use. Store at 4°C.

**Catch and Release<sup>®</sup> v2.0 Spin Columns, 50 columns** containing 0.5ml of prepacked IP capture resin. Store at 4°C

**Catch and Release<sup>®</sup> Capture Tubes, 100**, 2ml reservoir tubes.

**FOR RESEARCH USE ONLY  
NOT FOR USE IN HUMANS**

---

#### Kit Description

**Quantity:** 50 Immunoprecipitations per kit.

**Storage and Stability:** All components to be stored at 4°C except the Catch and Release<sup>®</sup> Capture Tubes which are stored at room temperature. Components are stable for 6 months from date of shipment.

**Use:** This kit allows for quick and reproducible immunoprecipitation (IP) of phosphorylated tyrosine residues on proteins by using a spin column. The system is more reproducible than regular IP's, which are problematic with regards to washing the protein A/G agarose without disrupting the agarose bed. The binding of the antibody/antigen complex in Catch and Release<sup>®</sup> is reversible, and elution of the immune complex can occur with native or denaturing buffers. This kit is only suitable for use with anti-Phosphotyrosine, recombinant clone 4G10<sup>®</sup>, Catalog # 05-777. **This kit can not be used with any other antibody other than the one listed above.** Please read the attached protocol before use.

**\*\*\*Native 4G10<sup>®</sup> antibody, Catalog # 05-321, CANNOT be used with this kit.\*\*\***

---

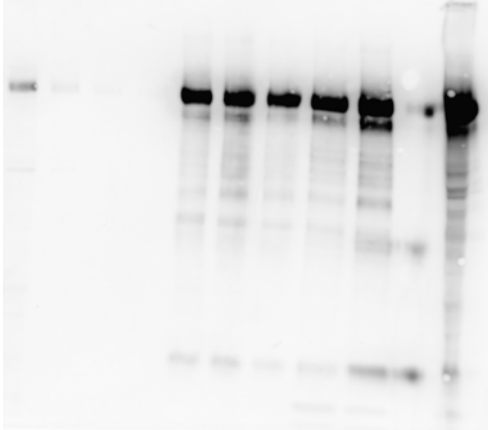
#### **Application References:**

1. Li, *et al.*, *J. Biol. Chem.* **280**: 6036-6046, 2005.
2. Sastri, *et al.*, *PNAS* **102**: 349-354, 2005.
3. Digicaylioglu, *et al.*, *PNAS* **101**: 9855-9860, 2004.
4. Song, *et al.*, *Mol. Endocrinol.* **18**: 70-85, 2004.

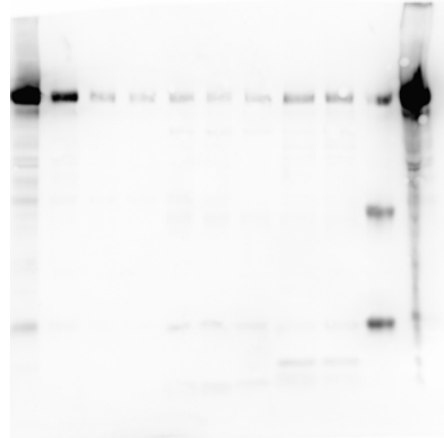
### Quality Control Testing

Catch and Release<sup>®</sup> Phosphotyrosine, clone 4G10<sup>®</sup> Immunoprecipitation Kit

**A.**



**B.**



Catch and Release<sup>®</sup> Spin columns and protocol were used with the Non-denaturing Elution Buffer to immunoprecipitate tyrosine phosphorylated proteins. EGF-stimulated A431 cell lysate (Catalog # 12-302) was mixed with A. Anti-Phosphotyrosine, recombinant clone 4G10<sup>®</sup> (Catalog # 05-777) or B. Normal, Mouse IgG (Catalog # 12-371) as a negative control for 1 hour at room temperature. Samples from each fraction were run on an SDS-PAGE gel and immunoblotted with anti-Phosphotyrosine, recombinant clone 4G10<sup>®</sup> (Catalog # 05-777). Bands indicate tyrosine phosphorylated proteins Lane 1: Flow through, Lane 2: Wash 1, Lane 3: Wash 2, Lane 4: Wash 3, Lane 5: 1X Non-denaturing Elution Buffer, Lane 6: 2X Non-denaturing Elution Buffer, Lane 7: 4X Non-denaturing Elution Buffer, Lane 8: 1X Denaturing Elution Buffer, Lane 9: Boiled beads (to detect complex remaining following elutions), Lane 10: 1µg anti-Phosphotyrosine, recombinant clone 4G10<sup>®</sup>, (Panel A) or 1µg Normal, Mouse IgG (Panel B), Lane 11: 30µg EGF-stimulated A431 lysate.