
Certificate of Analysis

Catch & Release® v2.0 High Throughput (HT) Immunoprecipitation Assay Kit – 96 well Catalog # 17-501 Lot # 30526

Kit Components

Antibody Capture Affinity Ligand, Catalog #20-216. One vial containing **60µg** Antibody Capture Affinity Ligand in **600µl** PBS containing 2mM PMSF and 10% glycerol. Store at 4°C.

Catch and Release® v2.0 HT Affinity resin, Catalog #16-250. One vial containing **1.2ml** (20% w/v) of IP capture resin in suspension. Store at 4°C.

Catch and Release® 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. Store at 4°C.

Catch and Release® 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® 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® 96 well filter plate, 1 plate. Store at room temperature.

Catch and Release® Capture 96 well plate, 1 plate. Store at room temperature.

10X PBS, Catalog #20-281. One bottle containing **24ml** 10X PBS. Dilute to 1X with water prior to use. Store at 4°C.

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

Kit Description

Quantity: 96 Immunoprecipitations per kit.

Storage and Stability: All components to be stored at 4°C. Components are stable for 6 months from date of shipment.

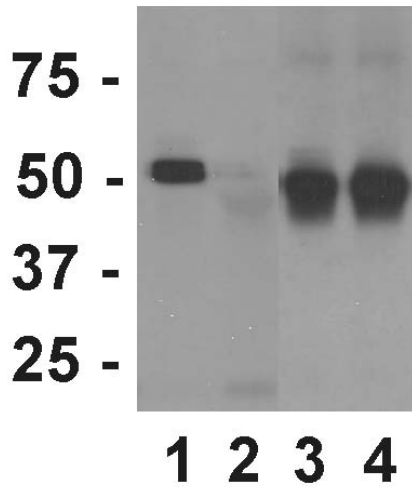
Use: This kit allows for quick and reproducible immunoprecipitation (IP) by using a 96 well filter plate. The system is more reproducible than regular IP's, which are problematic with regards to washing the protein A/G agarose without disrupting the agarose bed. The binding of the antibody/antigen complex in Catch and Release® is reversible, and elution of the immune complex can occur with native or denaturing buffers. The system has been tested successfully with rabbit, mouse, sheep and goat antibodies. IP using human IgG₁₋₄ should be suitable. IP using chicken antibodies or human IgA, IgD, IgE or IgM is not recommended with this kit. Please read the enclosed product manual before use.

References:

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

Quality Control Testing

Catch and Release[®] High Throughput (HT) Immunoprecipitation Data



Catch and Release[®] v2.0 High Throughput (HT) Immunoprecipitation Assay Kit and protocol were used with the Denaturing Elution Buffer to immunoprecipitate Akt1. 100µg HEK 293 cell lysate was mixed with 2µg anti-Akt1/PKB α (Catalog # 07-416) (Lane 1) or 2µg of normal, rabbit IgG (Catalog # 12-370) (Lane 2) as a negative control for 1 hour at room temperature. Samples from each IP fraction were run on an SDS-PAGE gel and immunoblotted with anti-Akt/PKB (Catalog # 05-591). Lanes 3 and 4 were only probed with anti-rabbit IgG, HRP conjugate (Catalog # 12-348) to show equal amounts of the IP fractions were loaded per lane.