
Certificate of Analysis

Catch and Release[®] v2.0
(Reversible Immunoprecipitation System)
Catalog # 17-500
Lot # 0703053675

Kit Components

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

Catch and Release[®] Wash Buffer, 10X, Catalog # 20-210. One vial containing **15 mL** of 10X buffer, pH 7.4 containing the following detergents: 10% NP-40, 2.5% deoxycholic acid and 150 mM 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[®] Non-denaturing Elution Buffer, 4X, Catalog # 20-209. One vial containing **10 mL** of 4X PBS-based IP Elution Buffer. Store at 4°C.

Catch and Release[®] Denaturing Elution Buffer, 1X, Catalog # 20-284. One vial containing **4 mL** 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[®] v2.0 Spin Columns, Catalog # 20-285. **50 columns** containing 0.5ml of prepacked IP capture resin. Store at 4°C

Catch and Release[®] Capture Tubes, 100, 2mL reservoir tubes.

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

Kit Description

Quantity: 50 Immunoprecipitations per kit.

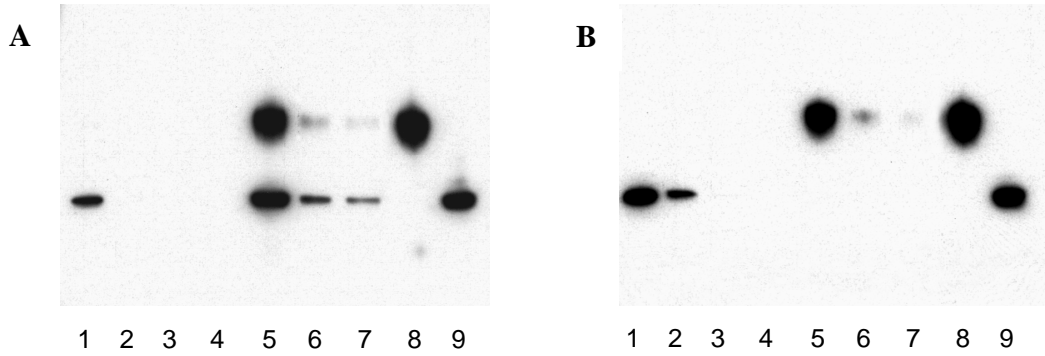
Storage and Stability: All components to be stored at 4°C except the Catch and Release[®] Capture Tubes which are stored at room temperature. Components are stable for 6 months from date of shipment.

Use: This kit allows for quick and reproducible immunoprecipitation (IP) 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[®] 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.

Application References:

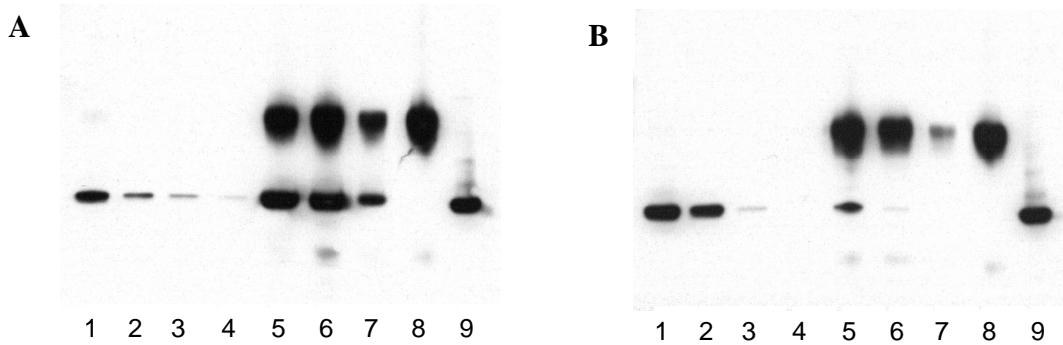
1. Li, *et al.*, *J. Biol. Chem.* **280**: 6036-6046, 2005.
2. Sastri, *et al.*, *PNAS* **102**: 349-354, 2005.
3. Digicaylioglu, *et al.*, *PNAS* **101**: 9855-9860, 2004.
4. Song, *et al.*, *Mol Endocrinol.* **18**: 70-85, 2004.

Quality Control Testing



Catch and Release[®] with Denaturing Elution Buffer:

Catch and Release[®] columns and protocol were used with the Denaturing Elution Buffer to immunoprecipitate cdk2. HeLa nuclear extract was mixed with A. Anti-cdk2 (Catalog # 06-505) or B. normal, rabbit IgG as a negative control for 1 hour at room temperature. Samples from each fraction were run on an SDS-PAGE gel and immunoblotted. The upper band is the heavy chain of IgG and the lower band is cdk2. Lane 1: flow through; Lane 2: wash 1; Lane 3: wash 2; Lane 4: wash 3; Lane 5: elution 1; Lane 6: elution 2; Lane 7: elution 3; Lane 8: Anti-cdk2; Lane 9: HeLa nuclear extract.



Catch and Release[®] with Non-denaturing Elution Buffer:

Catch and Release[®] columns and protocol were used with the Non-denaturing Elution Buffer to immunoprecipitate cdk2. HeLa nuclear extract was mixed with A. Anti-cdk2 (Catalog # 06-505) or B. normal, rabbit IgG as a negative control for 1 hour at room temperature. Samples from each fraction were run on an SDS-PAGE gel and immunoblotted. The upper band is the heavy chain of IgG and the lower band is cdk2. Lane 1: flow through; Lane 2: wash 1; Lane 3: wash 2; Lane 4: wash 3; Lane 5: elution 1; Lane 6: elution 2; Lane 7: elution 3; Lane 8: Anti-cdk2; Lane 9: HeLa nuclear extract.