

Applications	Reactivity	Sensitivity	MW (kDa)	Isotype
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IP	H	Endogenous	175	Mouse IgG2b
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**Applications Key:** IP=Immunoprecipitation

**Reactivity Key:** H=Human

Species cross-reactivity is determined by western blot. Species enclosed in parentheses are predicted to react based on 100% sequence homology.

## Protocols

### Immunoprecipitation Protocol / (For Analysis By Western Immunoblotting)

For **shorter assay times** please try our [Immunoprecipitation Protocol Utilizing Magnetic Separation / \(For Analysis By Western Immunoblotting\)](#).

#### A. Solutions and Reagents

**NOTE:** Prepare solutions with Milli-Q or equivalently purified water.

- 1X Phosphate Buffered Saline (PBS)
- 1X Cell Lysis Buffer:** ([#9803](#)) 20 mM Tris (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM Sodium pyrophosphate, 1 mM  $\beta$ -glycerophosphate, 1 mM  $\text{Na}_3\text{VO}_4$ , 1  $\mu\text{g/ml}$  Leupeptin  
**NOTE:** Add 1 mM PMSF immediately prior to use.
- Protein A or G Agarose Beads:** (Protein A [#9863](#)) Please prepare according to manufacturer's instructions. Use Protein A for rabbit IgG pull down and Protein G for mouse IgG pull down.
- 3X SDS Sample Buffer:** ([#7722](#)) 187.5 mM Tris-HCl (pH 6.8 at 25 °C), 6% w/v SDS, 30% glycerol, 150 mM DTT, 0.03% w/v bromophenol blue

#### B. Preparing Cell Lysates

- Aspirate media. Treat cells by adding fresh media containing regulator for desired time.
- To harvest cells under nondenaturing conditions, remove media and rinse cells once with ice-cold PBS.
- Remove PBS and add 0.5 ml ice-cold 1X cell lysis buffer to each plate (10 cm) and incubate the plates on ice for 5 minutes.
- Scrape cells off the plates and transfer to microcentrifuge tubes. Keep on ice.
- Sonicate samples on ice three times for 5 seconds each.
- Microcentrifuge for 10 minutes at 14,000 X g, 4 °C, and transfer the supernatant to a new tube. If necessary, lysate can be stored at -80 °C.

#### C. Immunoprecipitation

**Optional:** It may be necessary to perform a lysate pre-clearing step to reduce non-specific binding to the Protein A/G agarose beads (See section below).

- Take 200  $\mu\text{l}$  cell lysate and add primary antibody. Incubate with gentle rocking overnight at 4 °C.
- Add either protein A or G agarose beads (20  $\mu\text{l}$  of 50% bead slurry). Incubate with gentle rocking for 1–3 hours at 4 °C.
- Microcentrifuge for 30 seconds at 4 °C. Wash pellet five times with 500  $\mu\text{l}$  of 1X cell lysis buffer. Keep on ice during washes.
- Resuspend the pellet with 20  $\mu\text{l}$  3X SDS sample buffer. Vortex, then microcentrifuge for 30 seconds.
- Heat the sample to 95–100 °C for 2–5 minutes and microcentrifuge for 1 minute at 14,000 X g.

6. Load the sample (15–30 µl) on SDS-PAGE gel (12–15%).
7. Analyze sample by Western blotting (see Western Immunoblotting Protocol: [Western BSA](#), [Western Milk](#)).

### Cell Lysate Pre-Clearing (Optional)

1. Take 200 µl cell lysate and add to either Protein A or G agarose beads (20 µl of 50% bead slurry).
2. Incubate at 4 °C for 30 – 60 minutes.
3. Spin for 10 minutes at 4 °C. Transfer the supernatant to a fresh tube.
4. Proceed to step 1 of Immunoprecipitation.

**NOTE:** For proteins with molecular weights of 50 kDa, we recommend using [Mouse Anti-Rabbit IgG \(Light-Chain Specific\) \(L57A3\) mAb #3677](#) or [Mouse Anti-Rabbit IgG \(Conformation Specific\) \(L27A9\) mAb #3678](#) as a secondary antibody to minimize masking produced by denatured heavy chains. For proteins with molecular weights of 25 kDa, [Mouse Anti-Rabbit IgG \(Conformation Specific\) \(L27A9\) mAb #3678](#) is recommended.

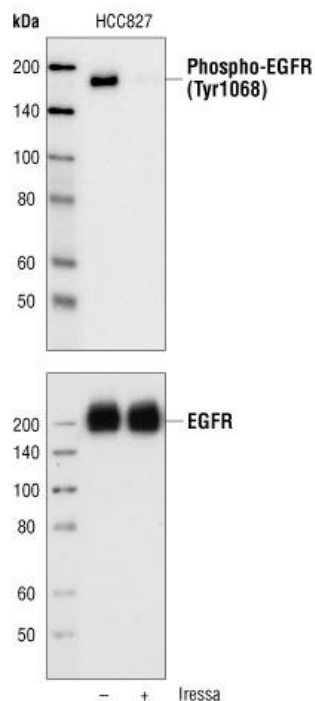
### Specificity / Sensitivity

EGF Receptor (EGFR1) Mouse mAb (IP Specific) specifically immunoprecipitates endogenous EGF receptors from various cell lysates. This antibody does not cross-react with other EGF receptor family members.

### Source / Purification

Monoclonal antibody is produced by immunizing animals with a recombinant protein corresponding to the extracellular domain of human EGF receptor.

### Western Blotting



Western blot analysis of EGF Receptor (EGFR1) Mouse mAb (IP Specific) immunoprecipitated samples from Iressa-treated and untreated HCC827 cell lysates, using Phospho-EGF Receptor (Tyr1068) Antibody (#2234) (upper) and EGF Receptor Antibody (#2232) (lower).