

#4405 Store at -20°C

EGF Receptor (15F8) Rabbit mAb (IHC Preferred)

100 µl
 (20 sections)



Orders ■ 877-616-CELL (2355)
 orders@cellsignal.com
Support ■ 877-678-TECH (8324)
 info@cellsignal.com
Web ■ www.cellsignal.com

rev. 10/17/07

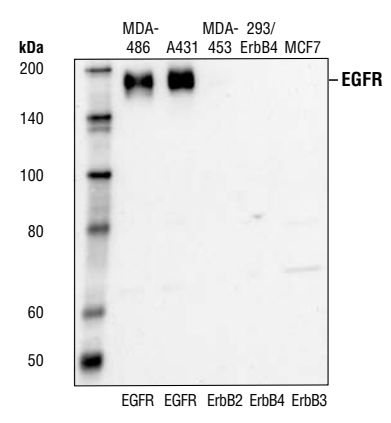
This product is for *in vitro* research use only and is not intended for use in humans or animals.

Applications W, IHC-P, IF-P	Species Cross-Reactivity* H	Molecular Wt. 175 kDa	Source Rabbit	Isotype IgG
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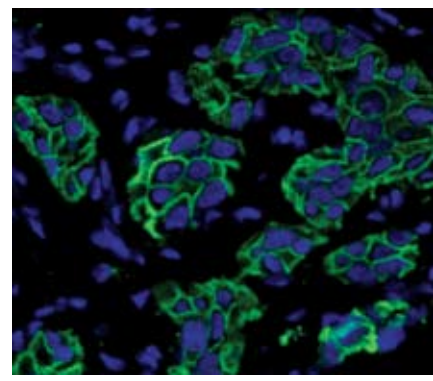
Background: Epidermal growth factor (EGF) receptor is a 170 kDa tyrosine kinase. Ligand binding results in receptor dimerization, autophosphorylation, activation of downstream signaling and lysosomal degradation (1,2). Phosphorylation of Tyr845 in the kinase domain is implicated in stabilizing the activation loop, maintaining the enzyme in an active state and providing a binding surface for substrate proteins (3,4). c-Src is involved in phosphorylation of Tyr845 (5). Phospho-tyrosine 992 is a direct binding site for the PLCγ SH2 domain, resulting in activation of PLCγ-mediated downstream signaling (6). Phosphorylation of Tyr1045 creates a major docking site for c-Cbl (7). Binding of c-Cbl to the activated EGFR leads to receptor ubiquitination and degradation (8). Phospho-Tyr1068 of activated EGFR is a direct binding site for Grb2 (9). Phospho-tyrosine 1148 and 1173 provide a docking site for SHC (2). Both sites are involved in the activation of MAP kinase signaling. Phosphorylation of EGFR on serine and threonine residues attenuates EGFR kinase activity. Serines 1046 and 1047 in the carboxy-terminal region of EGFR are sites phosphorylated by CaM kinase II. Mutations to either serine 1046 or 1047 upregulate tyrosine autokinase activity of EGFR (10).

Specificity/Sensitivity: EGF Receptor (15F8) Rabbit mAb (IHC Preferred) detects endogenous levels of EGF receptor proteins. The antibody does not recognize other proteins of the ErbB family.

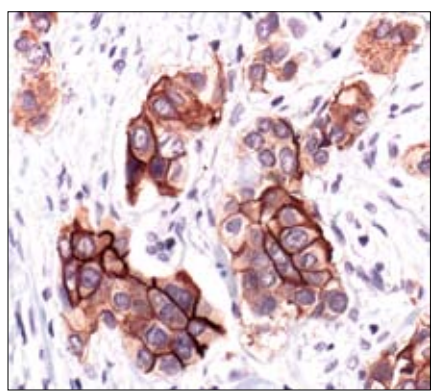
Source/Purification: Monoclonal antibodies are produced by immunizing rabbits with a synthetic peptide (KLH-coupled) corresponding to residues near the carboxy terminus of human EGF receptor.



Western blot analysis of various cell lysates using EGF Receptor (15F8) Rabbit mAb (IHC Preferred). This antibody does not cross-react with ErbB2, ErbB3 or ErbB4 expressed in MDA-453, MCF7 and 293/ErbB4 cells respectively.



Immunofluorescent analysis of paraffin-embedded human breast carcinoma, using EGF Receptor (15F8) Rabbit mAb (IHC Preferred) (green) and DAPI (blue).



Immunohistochemical analysis of paraffin-embedded human breast carcinoma, using EGF Receptor (15F8) Rabbit mAb (IHC Preferred).

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at -20°C. Do not aliquot the antibody.

***Species cross-reactivity is determined by Western blot.**

Recommended Antibody Dilutions:

Western Blotting	1:1000
Immunohistochemistry (Paraffin)	1:50
IHC Protocol: Unmasking buffer/wash buffer	EDTA/TBST
Immunofluorescence (IF-P)	1:50

Companion Products:

- Phospho-EGF Receptor (Tyr1068) (1H12) Mouse mAb #2236
- Phospho-EGF Receptor (Tyr845) Antibody #2231
- Phospho-EGF Receptor (Tyr1068) Antibody #2234
- EGF Receptor Antibody #2232
- EGF Receptor (1F4) Mouse mAb #2239
- Phototope®-HRP Western Blot Detection System, Anti-rabbit IgG, HRP-linked Antibody #7071
- Anti-rabbit IgG, HRP-linked Antibody #7074
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder Detection Pack #7727
- 20X LumiGLO® Reagent and 20X Peroxide #7003

Background References:

- Hackel, P.O. et al. (1999) *Curr. Opin. Cell Biol.* 11, 184–189.
- Zwick, E. et al. (1999) *Trends Pharmacol. Sci.* 20, 408–412.
- Cooper, J.A. and Howell, B. (1993) *Cell* 73, 1051–1054.
- Hubbard, S.R. et al. (1994) *Nature* 372, 746–754.
- Biscardi, J.S. et al. (1999) *J. Biol. Chem.* 274, 8335–8343.
- Emler, D.R. et al. (1997) *J. Biol. Chem.* 272, 4079–4086.
- Levkowitz, G. et al. (1999) *Mol. Cell* 4, 1029–1040.
- Ettenberg, S.A. et al. (1999) *Oncogene* 18, 1855–1866.
- Rojas, M. et al. (1996) *J. Biol. Chem.* 271, 27456–27461.
- Feinmesser, R.L. et al. (1999) *J. Biol. Chem.* 274, 16168–16173.

IMPORTANT: For Western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

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Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry IC—Immunocytochemistry IF—Immunofluorescence
Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken X—Xenopus
 Species enclosed in parentheses are predicted to react based on 100% sequence homology.

F—Flow cytometry E—ELISA D—DELFIATM
 Z—zebra fish B—bovine All—all species expected

Western Immunoblotting Protocol (Primary Antibody Incubation in BSA)

For Western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

A Solutions and Reagents

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

- 1X Phosphate Buffered Saline (PBS)
- 1X SDS Sample Buffer:** 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
- Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
- 10X Tris Buffered Saline (TBS):** To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
- Nonfat Dry Milk (weight to volume [w/v])
- Blocking Buffer:** 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).
- Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
- Bovine Serum Albumin (BSA)
- Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µl Tween-20 (100%).
- Phototope®-HRP Western Blot Detection System #7071:** Includes biotinylated protein ladder, secondary anti-rabbit (#7074) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO® chemiluminescent reagent and peroxide.
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder Detection Pack #7727
- Blotting Membrane:** This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

B Protein Blotting

A general protocol for sample preparation is described below.

- Treat cells by adding fresh media containing regulator for desired time.
- Aspirate media from cultures; wash cells with 1X PBS; aspirate.
- Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
- Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
- Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
- Microcentrifuge for 5 minutes.
- Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

NOTE: CST recommends loading prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.

- Electrotransfer to nitrocellulose or PVDF membrane.

C Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm²) of membrane; for different sized membranes, adjust volumes accordingly.

- (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
- Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.

D Detection of Proteins

- Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO®, 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

NOTE: LumiGLO® substrate can be further diluted if signal response is too fast.

- Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

NOTE: Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO® incubation and declines over the following 2 hours.

Immunohistochemistry Protocol (Paraffin)

***IMPORTANT:** See product data sheet for the appropriate wash buffer and antigen unmasking procedure. **IHC Protocol:** Unmasking buffer/wash buffer.

A Solutions and Reagents

- Xylene
- Ethanol, anhydrous denatured, histological grade (100% and 95%)
- Deionized water (dH₂O)
- Hematoxylin (optional)
- *Wash Buffer:**
 - PBST:** 1X PBS/0.1% Tween-20 (wash buffer): To prepare 1 L add 100 ml 10X PBS to 900 ml dH₂O. Add 1ml Tween-20 and mix.
10X Phosphate Buffered Saline (PBS): To prepare 1 L add 80 g sodium chloride (NaCl), 2 g potassium chloride (KCl), 14.4 g sodium phosphate, dibasic (Na₂HPO₄) and 2.4 g potassium phosphate, monobasic (KH₂PO₄) to 1 L dH₂O. Adjust pH to 7.4.
 - TBST:** 1X TBS/0.1% Tween-20 (wash buffer): To prepare 1 L add 100 ml 10X TBS to 900 ml dH₂O. Add 1 ml Tween-20 and mix.
10X Tris Buffered Saline (TBS): To prepare 1 L add 24.2 g Trizma® base (C₄H₁₁NO₃) and 80 g sodium chloride (NaCl) to 1 L dH₂O. Adjust pH to 7.6 with concentrated HCl.
- *Antigen Unmasking Solution:**
 - Citrate:** 10 mM Sodium Citrate Buffer: To prepare 1 L, add 2.94 g sodium citrate trisodium salt dihydrate (C₆H₅Na₃O₇•2H₂O) to 1 L dH₂O. Adjust pH to 6.0.
 - EDTA:** 1 mM EDTA: To prepare 1 L add 0.372 g EDTA (C₁₀H₁₄N₂O₈Na₂•2H₂O) to 1 L dH₂O. Adjust pH to 8.0.
 - Alternative Unmasking: 10 mM Tris:** To prepare 1 L add 1.21 g Trizma® Base (C₄H₁₁NO₃) to 1 L dH₂O. Adjust pH to 10.0.
 - Pepsin:** 1 mg/ml in Tris-HCl pH 2.0.
- 3% Hydrogen Peroxide:** To prepare, add 10 ml 30% H₂O₂ to 90 ml dH₂O.
- Blocking Solution:** 5% horse serum or goat serum diluted in recommended wash buffer.
- Biotinylated secondary antibody.
- ABC Reagent:** (Vectastain ABC Kit, Vector Laboratories, Inc., Burlingame, CA) Prepare according to manufacturer's instructions 30 minutes before use.
- DAB Reagent or suitable substrate:** Prepare according to manufacturer's recommendations.

B Deparaffinization/Rehydration

NOTE: Do not allow slides to dry at any time during this procedure.

NOTE: Consult product data sheet for recommended wash buffer.

- Deparaffinize/hydrate sections:**
 - Incubate sections in three washes of xylene for 5 minutes each.
 - Incubate sections in two washes of 100% ethanol for 10 minutes each.
 - Incubate sections in two washes of 95% ethanol for 10 minutes each.
- Wash sections twice in dH₂O for 5 minutes each.

C *Antigen Unmasking

NOTE: Consult product data sheet for specific recommendation for the unmasking solution.

- For Citrate:** Bring slides to a boil in 10 mM sodium citrate buffer pH 6.0 then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench top for 30 minutes.
- For EDTA:** Bring slides to a boil in 1 mM EDTA pH 8.0 followed by 15 minutes at a sub-boiling temperature. No cooling is necessary.
- Alternate:** Bring slides to a boil in 10 mM Tris pH 10.0 followed by 10 minutes at a sub-boiling temperature. Cool slides on bench top for 30 minutes.
- For Pepsin:** Digest for 10 minutes at 37°C.

D Staining

- Wash sections in dH₂O three times for 5 minutes each.
- Incubate sections in 3% hydrogen peroxide for 10 minutes.
- Wash sections in dH₂O twice for 5 minutes each.

NOTE: Consult product data sheet for recommended wash buffer.

- Wash section in wash buffer for 5 minutes.
- Block each section with 100-400 µl blocking solution for 1 hour at room temperature.
- Remove blocking solution and add 100-400 µl diluted primary antibody to each section. (Dilute antibody in blocking solution.) Incubate overnight at 4°C.
- Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
- Add 100-400 µl secondary antibody, diluted in blocking solution per manufacturer's recommendation, to each section. Incubate 30 minutes at room temperature.
- If using ABC avidin/biotin method, make ABC reagent according to the manufacturer's instructions and incubate solution for 30 minutes at room temperature.
- Remove secondary antibody solution and wash sections three times with wash buffer for 5 minutes each.
- Add 100-400 µl ABC reagent to each section and incubate for 30 minutes at room temperature.
- Remove ABC reagent and wash sections three times in wash buffer for 5 minutes each.
- Add 100-400 µl DAB or suitable substrate to each section and monitor staining closely.
- As soon as the sections develop, immerse slides in dH₂O.
- If desired, counterstain sections in hematoxylin per manufacturer's instructions.
- Wash sections in dH₂O two times for 5 minutes each.
- Dehydrate sections:
 - Incubate sections in 95% ethanol two times for 10 seconds each.
 - Repeat in 100% ethanol, incubating sections two times for 10 seconds each.
 - Repeat in xylene, incubating sections two times for 10 seconds each.
- Mount coverslips.

Immunofluorescence Protocol

***IMPORTANT:** Please refer to the **APPLICATIONS** section on the front page of the data sheet to determine **IF THIS PRODUCT** is validated and approved for the specific protocol you will be using.

A Solutions and Reagents

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

- 1. 10X Phosphate Buffered Saline (PBS):** To prepare 1 L add 80 g sodium chloride (NaCl), 2 g potassium chloride (KCl), 14.4 g sodium phosphate, dibasic (Na₂HPO₄) and 2.4 g potassium phosphate, monobasic (KH₂PO₄) to 1 L dH₂O. Adjust pH to 7.4.
- Formaldehyde, 16%, methanol free, Polysciences, Inc. (cat# 18814), use fresh, store opened vials at 4°C in dark, dilute in PBS for use.
- Xylene
- Ethanol, anhydrous denatured, histological grade, 100% and 95%
- Distilled water (dH₂O)
- 1X PBS/0.3% Triton X-100 (PBS/Triton):** To prepare 1 L, add 100 ml 10X PBS to 900 ml dH₂O. Add 3 ml Triton X-100 and mix.
- 10 mM Sodium Citrate Buffer:** To prepare 1 L, add 2.94 g sodium citrate trisodium salt dihydrate (C₆H₅Na₃O₇•2H₂O) to 1 L dH₂O. Adjust pH to 6.0.
- 1X PBS, high salt (0.4M) (high salt PBS):** To prepare 1L, add 100 ml 10X PBS to 900 ml dH₂O. Add 23.38 g NaCl and mix.
- Fluorochrome-conjugated secondary antibody

NOTE: When using any primary or fluorochrome-conjugated secondary antibody for the first time, titrate the antibody to determine which dilution allows for the strongest specific signal with the least background for your sample.

10. Prolong[®] Gold Antifade Reagent (Invitrogen, Eugene, OR, Cat# P36930)

B Specimen Preparation

I. Cultured Cell Lines (IF-IC)

IMPORTANT: Please check the **APPLICATIONS** section of the data sheet to verify that this product is validated and approved for **(IF-IC)**.

NOTE: This general fixation protocol will work with most antibodies and cell lines. However, we recommend you try different IF/IC fixation methods (methanol or acetone alone, aldehyde alone, or combinations of these) to identify the optimal fixation protocol for each antibody and/or cell line.

NOTE: Cells should be grown, treated, fixed, and stained directly in multiwell plates, chamber slides, or on coverslips.

1. Rinse cells briefly in PBS.
2. Aspirate PBS, cover cells to a depth of 2-3 mm with 2-4% formaldehyde in PBS.

NOTE: Formaldehyde is toxic, use only in fume hood.

3. Allow cells to fix for 15 minutes at room temperature.
4. Aspirate fixative, rinse three times in PBS for 5 minutes each.

5. Methanol Permeabilization Step (if required, please refer to front page): After formaldehyde fixation, cover cells with ice-cold 100% methanol (use enough to cover cells completely to a depth of 3-5 mm, **DO NOT LET CELLS DRY**), incubate cells in methanol for 10 minutes in freezer, rinse in PBS for 5 minutes.

6. Proceed with Immunostaining section C.

II. Paraffin Sections (IF-P)

IMPORTANT: Please check the **APPLICATIONS** section of the data sheet to verify that this product is validated and approved for **(IF-P)**.

Deparaffinization/Rehydration:

1. Incubate sections in three washes of xylene for 5 minutes each.
2. Incubate sections in two washes of 100% ethanol for 10 minutes each.
3. Incubate sections in two washes of 95% ethanol for 10 minutes each.
4. Rinse sections twice in dH₂O for 5 minutes each.

Antigen Unmasking:

1. Place slides in room temperature 10 mM sodium citrate buffer pH 6.0.
2. Bring slides to boiling in sodium citrate buffer using water bath or microwave, then maintain at 95-99°C for 10 minutes.
3. Cool slides for 30 minutes on bench top.
4. Rinse sections in dH₂O three times for 5 minutes each.
5. Rinse sections in PBS for 5 minutes.
6. Proceed with Immunostaining section C.

III. Frozen/Cryostat Sections (IF-F)

IMPORTANT: Please check the **APPLICATIONS** section of the data sheet to verify that this product is validated and approved for **(IF-F)**.

NOTE: Fresh frozen/unfixed sections should be fixed immediately in 2-4% formaldehyde as follows to preserve signaling epitopes.

1. Cover sections with 2-4% formaldehyde in PBS

NOTE: Formaldehyde is toxic, use only in fume hood.

2. Allow cells to fix for 15 minutes at room temperature.
3. Rinse slides three times in PBS for 5 minutes each.

C Immunostaining

NOTE: All subsequent incubations should be carried out at room temperature unless otherwise noted in a humid light-tight box or covered dish/plate to prevent drying and fluorochrome fading.

1. Block specimen in 5% normal serum from same species as secondary antibody (eg. normal goat serum, normal donkey serum) in PBS/Triton for 60 minutes.
2. While blocking, prepare primary antibody by diluting as indicated on datasheet in PBS/Triton. You will need 50-100 µl per section, 25-50 µl per coverslip, chamber, or well (48 or 96 well plate).
3. Aspirate blocking solution, apply diluted primary antibody.

NOTE: For double-labeling, prepare a cocktail of mouse and rabbit primary antibodies at their appropriate dilutions in PBS/Triton.

4. Incubate overnight at 4°C.
5. Rinse three times in PBS for 5 minutes each.

OPTION: To decrease background stain, rinse in high salt PBS for two minutes between second and third PBS rinses. Be aware, this may reduce specific staining of some antibodies.

NOTE: If using primary antibodies directly conjugated with AlexaFluor[®] fluorochromes, then skip to step C8.

6. Incubate in fluorochrome-conjugated secondary antibody diluted in PBS/Triton for 1-2 hours at room temperature in dark.

NOTE: For double-labeling, prepare a cocktail of fluorochrome-conjugated anti-mouse and anti-rabbit primary antibodies at their appropriate dilutions in PBS/Triton.

7. Rinse in PBS/high salt PBS as in step 5.
8. Coverslip slides with Prolong[®] Gold Antifade Reagent or apply just enough to cover cells in multiwell plate.
9. Seal slides by painting around edges of coverslips with nail polish.
10. Examine specimens immediately using appropriate excitation wavelength, depending on fluorochrome for best results or store flat at 4°C in dark.