Store at –20°C

#3922

IGFBP-2 Antibody

☑ 100 µl
(10 Western mini-blots)

Cell Signaling TECHNOLOGY®

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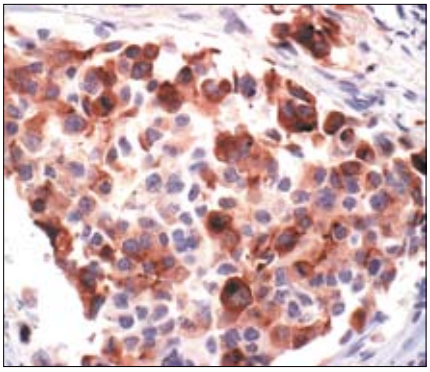
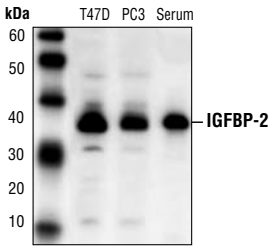
Rev. 07/13/06

Applications	Species Cross-Reactivity	Molecular Wt.	Source
W, IP, IHC-P	H	35 kDa	Rabbit

Background: Insulin-like growth factor-binding proteins (IGFBPs) play an integral role in modifying insulin-like growth factor (IGF) actions in a wide variety of cell types. This family contains six members that are structurally related but encoded by distinct genes. IGFBPs have a high affinity for IGFs. Some members of the IGFBP family have been consistently shown to inhibit IGF actions by preventing them from gaining access to the IGF receptors, while others potentiate IGF actions by facilitating the ligand-receptor interaction (1-3). IGFBP-2 is the second most abundant IGFBP in the circulation and is present in various other biological fluids and tissues of many vertebrate species. Serum IGFBP-2 levels are elevated in conditions such as shock, fasting, hypoxemia or after traumata, suggesting complex regulation of IGFBP-2 expression (4). IGFBP-2 is overexpressed in many malignancies and is often correlated with an increasingly malignant status of the tumor, pointing to a potential involvement of IGFBP-2 in tumorigenesis (5).



Immunohistochemical analysis of paraffin-embedded human breast carcinoma, showing cytoplasmic localization, using IGFBP-2 Antibody.



Western blot analysis of human serum and extracts from various cell lines, using IGFBP-2 Antibody.

Specificity/Sensitivity: IGFBP-2 Antibody detects endogenous levels of total IGFBP-2 protein. It does not cross-react with other IGFBP family members.

Source/Purification: Polyclonal antibodies are produced by immunizing rabbits with a synthetic peptide (KLH-coupled) corresponding to the residues at the carboxy-terminal sequence of human IGFBP-2. The antibodies are purified by protein A and peptide affinity chromatography.

Background References:

(1) Duan, C. (2002) *J. Endocrinol.* 175, 41–54.

(2) Sandhu, M.S. et al. (2002) *J. Natl. Cancer Inst.* 94, 972–980.

(3) Baxter, R.C. (2001) *Best Pract. Res. Clin. Endocrinol. Metab.* 15, 421–434.

(4) Noel, M. et al. (2001) *Curr. Opin. Clin. Nutr. Metab. Care* 4, 399–405.

(5) Zumkeller, W. (2001) *Mol. Pathol.* 54, 285–288.

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

Recommended Antibody Dilutions:

Western blotting	1:1000
Immunoprecipitation	1:50
Immunohistochemistry (Paraffin)	1:25

Companion Products:

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

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

Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **IC**—Immunocytochemistry **IF**—Immunofluorescence **F**—Flow cytometry **E**—ELISA **D**—DELFIA®

Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **X**—Xenopus **Z**—zebra fish **B**—bovine **All**—all species expected

Species enclosed in parentheses are predicted to react based on 100% sequence homology.

Western Immunoblotting Protocol (Primary Ab 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.

1. 1X Phosphate Buffered Saline (PBS)
2. **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
3. **Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
4. **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).
5. Nonfat Dry Milk (weight to volume [w/v])
6. **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%).
7. **Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
8. Bovine Serum Albumin (BSA)
9. **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%).
10. **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.
11. Prestained Protein Marker, Broad Range (Premixed Format) #7720
12. Biotinylated Protein Ladder Detection Pack #7727
13. **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.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. 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.
4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
5. Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
6. Microcentrifuge for 5 minutes.
7. 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.

8. Electrotransfer to nitrocellulose or PVDF membrane.

C Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm^2) of membrane; for different sized membranes, adjust volumes accordingly.

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
3. Wash three times for 5 minutes each with 15 ml of TBS/T.
4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
5. Wash three times for 5 minutes each with 15 ml of TBS/T.
6. 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.
7. Wash three times for 5 minutes each with 15 ml of TBS/T.

D Detection of Proteins

1. 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.

2. 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.

Immunoprecipitation Protocol / (For Analysis By Western Immunoblotting)

A Solutions and Reagents

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

1. 1X Phosphate Buffered Saline (PBS)
2. **1X Cell Lysis Buffer:** 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 β-glycerophosphate, 1 mM Na_3VO_4, 1 µg/ml Leupeptin

NOTE: CST recommends adding 1 mM PMSF before use*.

3. **Transfer Buffer:** 25 mM Tris base, 0.2 mM glycine, 20% methanol (pH 8.5)
4. **Protein A or G Agarose Beads:** (Can be stored for 2 weeks at 4°C.) Please prepare according to manufacturer's instructions. Use Protein A for rabbit IgG pull down and Protein G for mouse IgG pull down.
5. **3X SDS Sample Buffer:** 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

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

C Immunoprecipitation

1. Take 200 µl cell lysate and add primary antibody. Incubate with gentle rocking overnight at 4°C.
2. Add either protein A or G agarose beads (20 µl of 50% bead slurry). Incubate with gentle rocking for 1–3 hours at 4°C.
3. Microcentrifuge for 30 seconds at 4°C. Wash pellet five times with 500 µl of 1X cell lysis buffer. Keep on ice during washes.
4. Resuspend the pellet with 20 µl 3X SDS sample buffer. Vortex, then microcentrifuge for 30 seconds.
5. Heat the sample to 95–100°C for 2–5 minutes.
6. Load the sample (15–30 µl) on SDS-PAGE gel (12–15%).
7. Analyze sample by Western blotting (see Western Immunoblotting Protocol).

Immunohistochemistry Protocol for Paraffin Sections

A Solutions and Reagents

1. **10X Phosphate Buffered Saline (PBS):** 0.58 M sodium phosphate dibasic (Na_2HPO_4), 0.17 M sodium phosphate monobasic (NaH_2PO_4), 0.68 M NaCl. To prepare 1 liter of 10X PBS, combine 82.33 g Na_2HPO_4, 23.45 g $NaH_2PO_4 \bullet H_2O$ and 40 g NaCl. Adjust pH to 7.4.
2. **10 mM Sodium Citrate Buffer:** To prepare 1 liter, add 2.94 g sodium citrate to 1 liter distilled H_2O (dH_2O). Adjust pH to 6.0.
3. EDTA (optional)*
4. **1% Hydrogen Peroxide:** To prepare, add 10 ml 30% H_2O_2 to 290 ml dH_2O.
5. **Blocking Solution:** 5% horse serum or goat serum in PBS
6. **ABC Reagent:** (Vectastain ABC Kit, Vector Laboratories, Inc., Burlingame, CA) Prepare according to manufacturer's instructions 30 minutes before use.
7. **DAB Reagent:** Add 6.7 µl of 30% hydrogen peroxide to 10 ml dH_2O; add this mixture to 10 ml of 1 mg/ml DAB (diaminobenzidine tetrahydrochloride) in PBS, filter.

B Protocol

1. **Deparaffinize/hydrate sections:**
   - **1a.** Incubate sections in three washes of xylene for 5 minutes each.
   - **1b.** Incubate sections in two washes of 100% ethanol for 10 minutes each.
   - **1c.** Incubate sections in two washes of 95% ethanol for 10 minutes each.
2. Wash sections twice in dH_2O for 5 minutes each.
3. Wash sections in PBS for 5 minutes.
4. For antigen unmasking, heat sections in 10 mM sodium citrate buffer (pH 6.0) for 1 minute at full power followed by 9 minutes at medium power.* (Keep slides fully immersed in buffer and maintain temperature at or just below boiling.) Cool slides for 20 minutes after antigen unmasking.

* Alternatively in Step B4, use 1 mM EDTA (pH 8.0), which gives superior results for EGF receptor and HER2/ErbB2 antibodies. See the Tyrosine Kinases/Docking Proteins section of the catalog for these products.

5. Wash sections in dH_2O three times for 5 minutes each.
6. Incubate sections in 1% Hydrogen Peroxide for 10 minutes.
7. Wash sections in dH_2O three times for 5 minutes each.
8. Wash section in PBS for 5 minutes.
9. Block each section with 100–400 µl blocking solution for 1 hour at room temperature.
10. Remove blocking solution and add 100–400 µl diluted primary antibody to each section. (Dilute antibody in blocking solution.) Incubate overnight at 4°C.
11. Remove antibody solution and wash sections in PBS three times for 5 minutes each.
12. Add 100–400 µl secondary antibody, diluted in blocking solution, to each section. Incubate 30 minutes at room temperature.
13. If using ABC avidin/biotin method, make ABC reagent according to the manufacturer's instructions and incubate solution for 30 minutes at room temperature.
14. Remove secondary antibody solution and wash sections three times with PBS for 5 minutes each.
15. Add 100–400 µl ABC reagent to each section and incubate for 30 minutes at room temperature.
16. Remove ABC reagent and wash sections three times in PBS for 5 minutes each.
17. Add 100–400 µl DAB reagent to each section and monitor staining closely.
18. As soon as the section turns brown, immerse slides in dH_2O.
19. If desired, counterstain sections in hematoxylin for 10 seconds.
20. Wash sections in dH_2O two times for 5 minutes each.
21. **Dehydrate sections:**
    - **21a.** Incubate sections in 95% ethanol two times for 10 seconds each.
    - **21b.** Repeat in 100% ethanol, incubating sections two times for 10 seconds each.
    - **21c.** Repeat in xylene, incubating sections two times for 10 seconds each.
22. Mount coverslips.