Validation Report #029750

Validation Date: 07/01/14

Summary

Antigen	Insulin-Like Growth Factor 1 Receptor (IGF1R)
Catalog number	ABIN726575
Supplier	Bioss
Supplier catalog number	bs-0227R
Lot number	130322
Method validated	Western Blot
Laboratory	Alamo Laboratories Inc
Validation number	29750
Positive Control	MCF-7 cells – high expression
Negative Control	PC3 cells – low expression
Notes	Two strong bands were observed in the positive control, one at the expected molecular weight, and one slightly higher (which may represent a glycosylated or precursor form). Weaker bands of the same molecular weight appear in the negative control, which is consistent with the expected lower expression in PC3 cells. No other major bands are present in either positive or negative controls.

Full Methods

Primary Antibody
- Antigen: Insulin-Like Growth Factor 1 Receptor (IGF1R) (1:200 dilution)
- Catalog number: ABIN726575
- Supplier: Bioss
- Supplier catalog number: bs-0227R
- Lot number: 130322

Loading Control Antibody
- Antibody: Mouse Anti-Actin (1:6,000 dilution)
- Supplier: BD Transduction Laboratories
- Catalog number: 612657
- Lot number: N/A

Secondary Antibody
- Antibody: Goat Anti-Rabbit IgG (H + L)-HRP Conjugate (1:20,000 dilution)
- Supplier: Bio-Rad
- Catalog number: #170-6515
- Lot number: L170-6515

Controls
- Positive control: MCF-7 cell extract
- Negative control: PC-3 cell extract

Protocol
- Total protein extracts were boiled in 1X SDS Sample Buffer containing 1% SDS and 1.25% β-mercaptoethanol at 95°C for 5 min prior to loading.
- 46 µg of boiled extracts were loaded and resolved on a 8-16% SDS-polyacrylamide gel.
- The Spectra Multicolor Broad Range (Thermo Scientific, Cat # 26634) were used as molecular mass markers.
- Proteins were transferred onto PVDF membrane by wet transfer and protein transfer was confirmed with Ponceau-S staining.
- The PVDF membrane was incubated with 25 mL of blocking buffer [Tris Buffered Saline, pH 7.4 plus 0.1% TW20 (TBST)] containing 5% (W/V) non-fat dry milk at room temperature for 1 h.
- The membrane was rinsed with TBST once.
- The membrane was immersed with the protein side up in the primary antibody solution (anti-IGF1R; 1:200) in TBST containing 5% (W/V) non-fat dry milk and incubated for 16 hours at 4°C.
- The membrane was rinsed in TBST three times for 5 min each.
- The membrane was incubated in the HRP-conjugated secondary antibody solution (Goat anti-rabbit IgG-HRP; 1:20,000) in TBST containing 5% (W/V) non-fat dry milk and incubated for 1 hour at room temperature (~26°C) with gentle agitation.
- The membrane was rinsed three times with TBST for 5 min each.
- The membrane was rinsed in TBS twice for 30 s each.
- Signals were detected with ECL-2 Substrate. The blot was scanned for 300 s.
- The membrane was rinsed three times with TBST, then incubated in Acidic Glycine Stripping Buffer at room temperature with gentle agitation for 3 times, 10 min each.
- The membrane was washed in TBST 2 times for 10 min each.
- Repeated Steps 5-12 with the loading control antibody (anti-Actin; 1:6,000) and its matching secondary antibody (Goat anti-rabbit IgG-HRP; 1:20,000).

Experimental Notes
- No challenges noted.

Figures

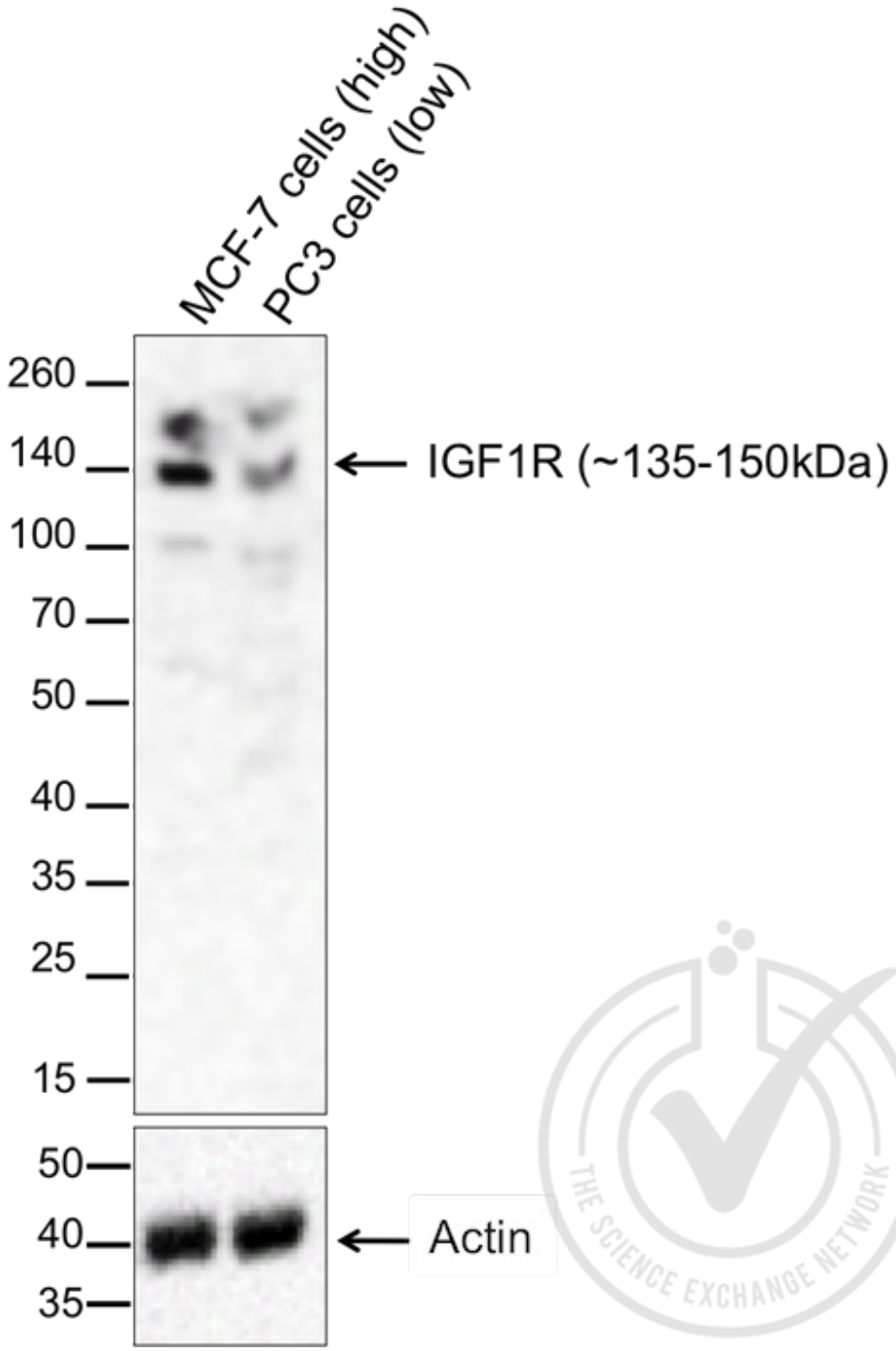

Figure 1. Western blot of lysates from MCF7 cells (Lane 1) and PC-3 cells (Lane 2) probed with anti-IGF1R (upper panel) or with anti-Actin for loading control (lower panel).