Validation Report #029817

Validation Date: 09/18/14

Summary

Antigen	Epidermal Growth Factor Receptor (EGFR)
Catalog number	ABIN98862
Supplier	antibodies-online
Lot number	19538
Method validated	Western Blot
Laboratory	ADS Biosystems Inc
Validation number	029817
Positive Control	A549 cells
Negative Control	MCF-7 cells
Notes	A strong specific band was observed in the positive control at the expected size (~175 kDa) that is not observed in the negative control.

Full Methods

Primary Antibody
- Antigen: Epidermal Growth Factor Receptor (EGFR)
- Catalog number: ABIN98862
- Supplier: antibodies-online
- Lot number: 19538
- Dilution: 1:1,000

Loading Control Antibody
- Antigen: Alpha-tubulin
- Supplier: Life Technologies
- Supplier catalog number: 322500
- Lot number: 1439416A
- Dilution: 1:10,000

Secondary Antibody
- Antibody: IRDye 680LT Goat Anti-Rabbit
- Catalogue number: 827-11081
- Supplier: LI-COR Biosciences
- Lot number: C30725-01
- Dilution: 1:10,000

Additional Information

Controls
- Lysates were prepared by ADS Biosystems following standard protocols and quality controlled for protein integrity on a regular basis

Protocol
- Lysates were mixed with NuPAGE® LDS Sample Buffer (Life Technologies NP0007) and NuPAGE® Sample Reducing Agent (Life Technologies NP0004) and denatured for 5 minutes at 90ºC.
- 40 µg of each lysate was electrophoresed on a Bolt 4-12% Bis-Tris Gel (Life Technologies BG04120BOX) and run in Bolt MOPS SDS Running Buffer (Life Technologies B0001) at 160 volts for 1 hour.
- Odyssey Western Protein Standard (LI-COR #928-40000) was run as a molecular weight standard.
- PVDF membrane was activated with methanol.
- Protein samples were transferred to activated PVDF membrane in a wet Bolt Transfer Apparatus (Life Technologies B1000) at room temperature for 1 hour at 20 volts (started at 230mA, ended at 110mA).
- The membrane was blocked in x LI-COR Odyssey WB block solution for 1 hour at room temperature.
- The membrane was incubated with the primary antibody diluted 1:1000 in x LI-COR Odyssey WB block solution incubated 2 hours at room temperature.
- The membrane was washed 4 x 5 minutes in 1 x PBS-T (PBS solution with 0.1% Tween 20).
- The membrane was incubated with IRDye® 800CW Goat anti-Mouse Secondary Antibody (Red) and IRDye 680LT Goat Anti-Rabbit Secondary Antibody (Green) from LI-COR (#827-11081, Lot #C30725-01), both 1:10,000 dilutions. Incubation was performed at room temperature for 45 minutes.
- The membrane was washed 4 x 5 minutes in 1 x PBS-T (PBS solution with 0.1% Tween 20).
- Proteins were detected using Odyssey machine scanning with green channel for loading control and red channel for potential LPL band.

Experimental Notes
- No experimental challenges noted.

Figures

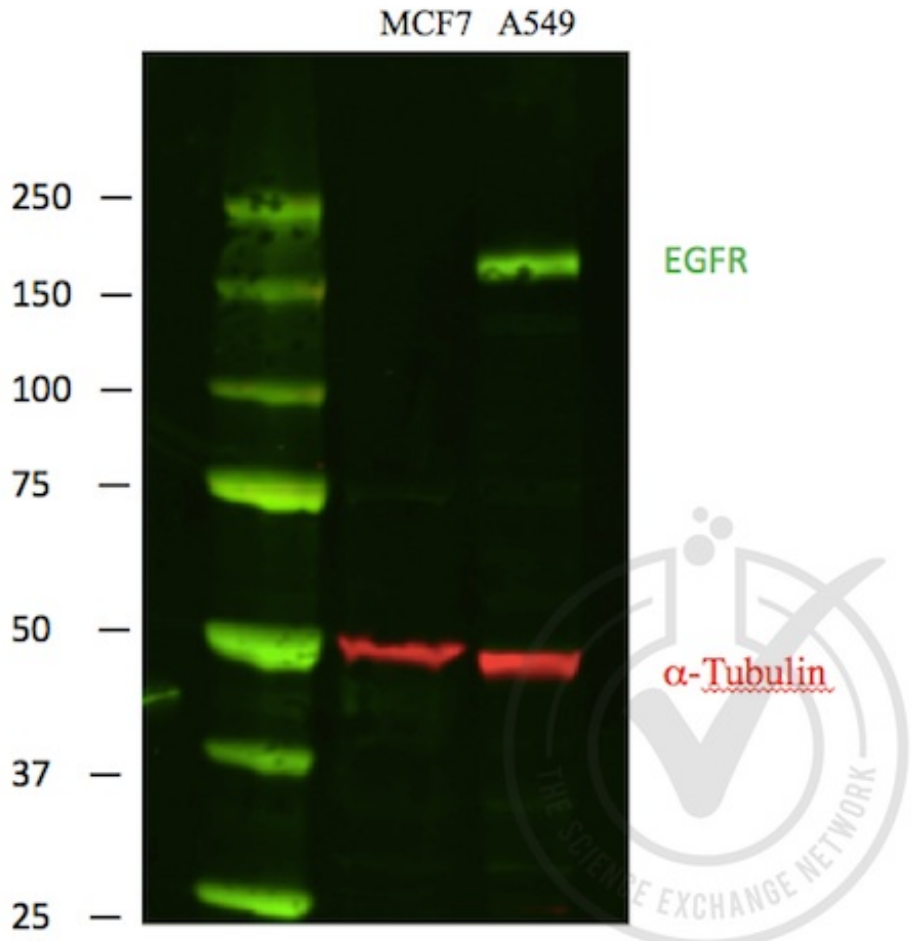

Figure 1: Scanned image of EGFR (Green) and loading control alpha-tubulin (Red) Western blot using LI-COR Odyssey Infrared Technology. First lane, protein molecular weight markers. Second lane, MCF-7 negative control lysate. Third lane, A549 positive control lysate.