ANTIBODY RESOURCE

ANTIBODY TESTING REPORT

SUMMARY

Antigen: EGFR (Uniprot# P00533)

Method tested: Western Blotting

Laboratory ID: LAB07

Project ID: AR116

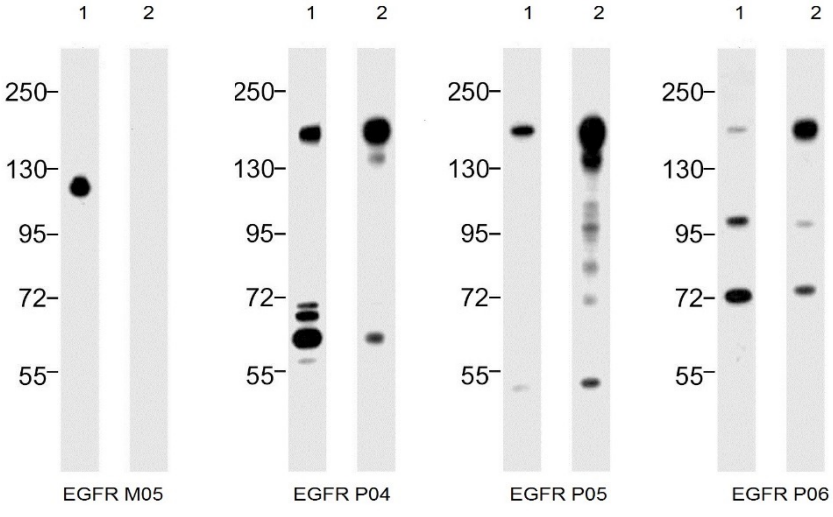
BACKGROUND

With thousands of proteins and often hundreds of associated antibodies, the selection of a specific antibody can be both time-consuming and expensive. Antibody Resource is spearheading a unique initiative designed to compare antibodies from numerous suppliers using identical samples/tissues and an identical protocol. In doing so, we hope to enable scientists to form an unrivalled opinion of which is the most suitable antibody for their research and in particular, which is going to require the least amount of optimisation, a process which can often take weeks or months.

For the purposes of the antibody comparison initiative, we select the best antibodies from each manufacturer and then compare them side-by-side using the same experimental conditions to provide a direct comparison. The antibodies are collected centrally, repackaged and given an internal reference ID prior to delivery to independent laboratories to ensure objective testing and to minimise bias.

Disclaimers: There is a possibility that results may vary between antibody lots. The results are indicative of the experimental conditions described within. Variations to this protocol may give alternative results.

RESULTS

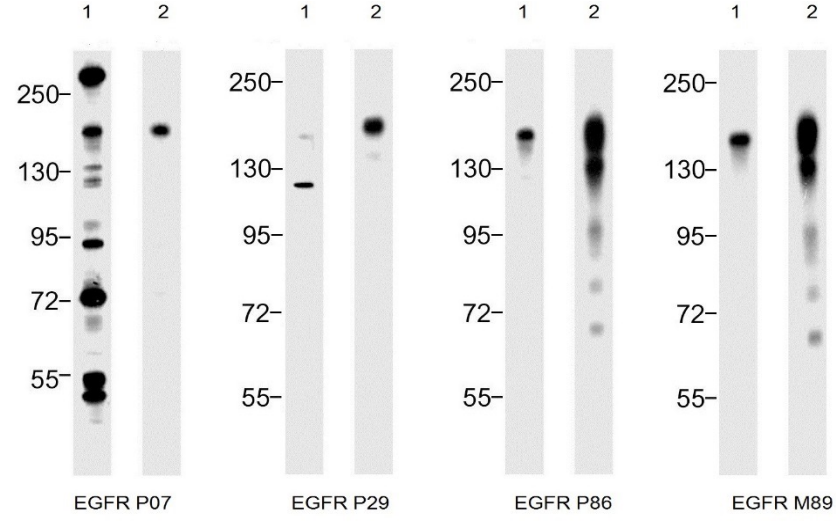


Western blot analysis of:-

(1) HeLa whole cell lysate

(2) A431 whole cell lysate

using various anti-EGFR antibodies (see Method for primary and secondary antibody details).



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METHOD

Antibodies

	Primary antibody	Secondary antibody
	EGFR M05 at 1/1000 (Supplier 06)	Anti-Mouse IgG (Fc specific)–Peroxidase antibody produced in goat (Sigma, Cat no. A0168) at 1/10,000
	EGFR P04 at 1/500 (Everest)	Peroxidase AffiniPure Bovine Anti-Goat IgG (H+L) (Jackson ImmunoResearch Labs Inc., Cat no. 805-035-180) at 1/10,000
	EGFR P05 at 1/500 (Everest)	Peroxidase AffiniPure Bovine Anti-Goat IgG (H+L) (Jackson ImmunoResearch Labs Inc., Cat no. 805-035-180) at 1/10,000
	EGFR P06 at 1/500 (Everest)	Peroxidase AffiniPure Bovine Anti-Goat IgG (H+L) (Jackson ImmunoResearch Labs Inc., Cat no. 805-035-180) at 1/10,000
	EGFR P07 at 1/500 (Everest)	Peroxidase AffiniPure Bovine Anti-Goat IgG (H+L) (Jackson ImmunoResearch Labs Inc., Cat no. 805-035-180) at 1/10,000
	EGFR P29 at 1/1000 (Fitzgerald)	Goat anti-Rabbit IgG (H+L) Cross Adsorbed Secondary Antibody, HRP conjugate (Thermo Scientific Pierce, Cat no. 31462) at 1/10,000
	**EGFR P86 at 1/1000 (Santa Cruz)**	Goat anti-Rabbit IgG (H+L) Cross Adsorbed Secondary Antibody, HRP conjugate (Thermo Scientific Pierce, Cat no. 31462) at 1/10,000
	**EGFR M89 at 1/1000 (Cell Signalling)**	Goat anti-Rabbit IgG (H+L) Cross Adsorbed Secondary Antibody, HRP conjugate (Thermo Scientific Pierce, Cat no. 31462) at 1/10,000

 = Component of the EGFR Superstarter Antibody Panel. See end of report for details.

Samples

Sample	Description
MW markers (Thermo Fisher Scientific, Cat no. 26619)	MW markers at 10, 17, 28, 36, 55, 72, 95, 130 and 250kDa.
HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysate at 20 µg/lane	Lane 1 - Test
A431 (Human epithelial carcinoma cell line) whole cell lysate at 20 µg/lane	Lane 2 - Test

Detection Kit

Clarity™ Western ECL Blotting Substrate (Bio-rad, Cat no: 170-5061, Lot number: 102030607).

PROTOCOL

Western Blotting was performed using Invitrogen's Novex® XCell SureLock® Mini-Cell electrophoresis system followed by semi dry transfer onto PVDF membranes using Bio-Rad's Trans-Blot® SD Semi-Dry Transfer Cell and visualized using X-ray film as follows:-

1. Samples (see table above) were incubated with 1X SDS Sample Buffer containing 2% SDS and 100mM DTT at 95^{o}C for 5 minutes prior to loading.
2. The samples were then loaded and resolved on an 8% SDS-polyacrylamide gel (see table above for amount protein per lane).
3. Proteins were transferred onto PVDF membrane by semi dry transfer and confirmed by amido black staining.
4. The immunoblot membrane was blocked in Tris buffered saline (TBS) containing Tween-20 (TBST) and 5% non-fat dry milk powder (blocking buffer) for 2 hours at room temperature with gentle agitation and then washed for 5 minutes in TBST.
5. The membrane was then immersed with the protein side up in the primary antibody solution (for details see table above) diluted in blocking buffer overnight at 4 ^{o}C with gentle agitation.
6. Following two washes for 5 minutes each and one wash for 10 minutes at room temperature with TBST, the membrane was incubated in the secondary antibody (for details see table above) diluted in blocking buffer for 1 hour at room temperature with gentle agitation.
7. The membrane was then washed three times for 5 minutes and then 8 minutes with TBST at room temperature.
8. After draining away excess TBST, signals were detected with the detection kit detailed above, the blots exposed on X-ray film and the final images obtained using PS software.

EXPERIMENTAL NOTES

EGFR P04, EGFR P05, EGFR P06, EGFR P07, EGFR P29, EGFR P86 and EGFR M89 demonstrated immunoreactivity with major bands at ~170kDa in both Human cell lines which is consistent with the expected MW of EGFR. Additional immunoreactive bands were observed in at least one and often both cell line lysates for these antibodies. These bands may represent isoforms of EGFR or they may be non-specific background as the banding pattern varied depending on the antibody and the cell lysate used.

Under these experimental conditions, bands at the expected MW were not observed for either cell lysate using EGFR M05 although the HeLa cell lysate did show immunoreactivity at ~120kDa.

SUPERSTARTER ANTIBODY PANELS

A panel of Superstar antibodies in trial sizes, to enable you to economically test the best antibodies, to determine which is going to be the best for your research project for only $287, €267, £98.

The EGFR Superstarter Antibody Panel consists of:
-1x 4267 Cell Signaling Technology (high ratings)
-1x sc-03 Santa Cruz Biotechnology (star performer)
-1x 06-847 Millipore (high ratings)
http://www.antibodyresource.com/superstars

Images of Superstar EGFR antibodies: