ANTIBODY RESOURCE

# ANTIBODY TESTING REPORT

## SUMMARY

**Antigen:** EGFR (Uniprot# P00533)

**Method tested:** Western Blotting

**Laboratory ID:** LAB06

**Project ID:** AR110

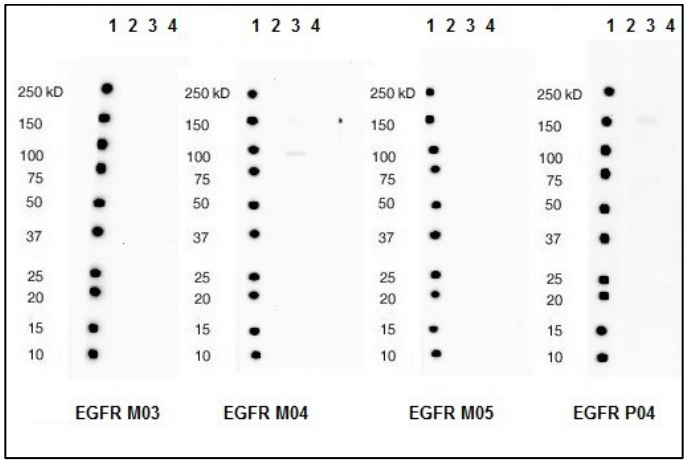
## BACKGROUND

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



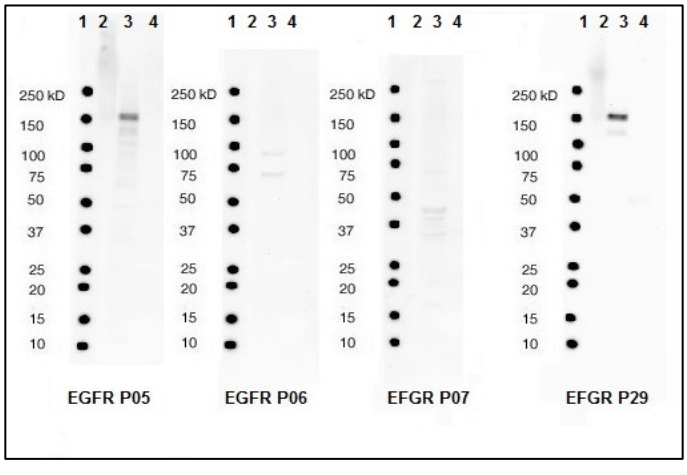

Western blot analysis of

(1) MW markers

(2) HeLa whole cell lysate

(3) A549 whole cell lysate

(4) Human lung tissue lysate using various anti-EGFR antibodies (see Method for primary and secondary antibody details). ECL exposure time was 300 seconds

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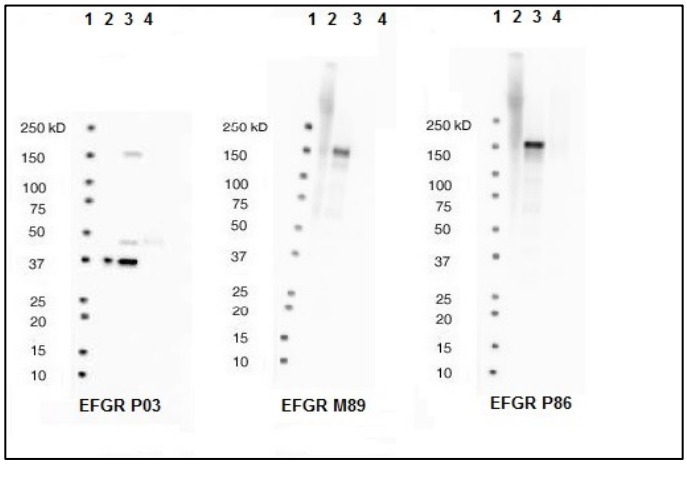


Western blot analysis of

(1) MW markers

(2) HeLa whole cell lysate

(3) A549 whole cell lysate

(4) Human lung tissue lysate using various anti-EGFR antibodies (see Method for primary and secondary antibody details). ECL exposure time was 60 seconds for the blots using EGFR P03 and EFGR M89 and 30 seconds for the EFGR P86 blot.

# METHOD

## Antibodies

| | Primary antibody | Secondary antibody |
|---|---|---|
| | **EGFR M03 at 1/1000 (Supplier 15)** | Goat anti-Mouse IgG (H+L) HRP conjugated antibody (Jackson ImmunoResearch Laboratories, Inc., Cat no. 115-035-062) at 1/10,000 |
| | **EGFR P03 at 1/1000 (Supplier 29)** | Goat anti-Rabbit HRP conjugated antibody (Aviva Systems Biology, Cat no. ASP00001) at 1/10,000 |
| | **EGFR M04 at 1/1000 (Supplier 07)** | Goat anti-Mouse IgG (H+L) HRP conjugated antibody (Jackson ImmunoResearch Laboratories, Inc., Cat no. 115-035-062) at 1/10,000 |
| | **EGFR M05 at 1/1000 (Supplier 06)** | Goat anti-Mouse IgG (H+L) HRP conjugated antibody (Jackson ImmunoResearch Laboratories, Inc., Cat no. 115-035-062) at 1/10,000 |
| | **EGFR P04 at 1/500 (Everest)** | Mouse anti-Goat IgG (H+L) HRP conjugated antibody (Jackson ImmunoResearch Laboratories, Inc., Cat no. 205-035-108) at 1/10,000 |
| | **EGFR P05 at 1/500 (Everest)** | Mouse anti-Goat IgG (H+L) HRP conjugated antibody (Jackson ImmunoResearch Laboratories, Inc., Cat no. 205-035-108) at 1/10,000 |
| | **EGFR P06 at 1/500 (Supplier 14)** | Mouse anti-Goat IgG (H+L) HRP conjugated antibody (Jackson ImmunoResearch Laboratories, Inc., Cat no. 205-035-108) at 1/10,000 |
| | **EGFR P07 at 1/500 (Supplier 14)** | Mouse anti-Goat IgG (H+L) HRP conjugated antibody (Jackson ImmunoResearch Laboratories, Inc., Cat no. 205-035-108) at 1/10,000 |
| | **EGFR P29 at 1/1000 (Fitzgerald)** | Goat anti-Rabbit HRP conjugated antibody (Aviva Systems Biology, Cat no. ASP00001) at 1/10,000 |
| | **EGFR P86 at 1/1000 (Santa Cruz)** | Goat anti-Rabbit HRP conjugated antibody (Aviva Systems Biology, Cat no. ASP00001) at 1/10,000 |
| | **EGFR M89 at 1/1000 (Cell Signalling)** | Goat anti-Rabbit HRP conjugated antibody (Aviva Systems Biology, Cat no. ASP00001) at 1/10,000 |

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

## Samples

| Sample | Description |
|---|---|
| MW markers (Bio-Rad, Cat no. 161-0376) | Lane 1 - MW markers at 10, 15, 20, 25, 37, 50, 75, 100, 150 and 250kDa. |
| HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysate at 25 µg/lane; (Aviva Systems Biology, Cat no. AHL006) | Lane 2 - Test |
| A549 (Human epithelial cells from lung carcinoma) whole cell lysate at 25 µg/lane; (Aviva Systems Biology, Cat no. AHL018) | Lane 3 - Test |
| Human lung tissue lysate at 25 µg/lane | Lane 4 - Test |

# PROTOCOL

Western Blotting was performed using BioRad's V3 Workflow System, comprising of a Mini PROTEAN® 3 Dodeca cell, a TransBlot®Turbo™transfer system and ChemiDoc XRS system.

1. Samples (see table above) were incubated with 4X SDS Sample Buffer at 95-99ºC for 3-4 minutes prior to loading. The ratio of samples to sample buffer was adjusted so that the samples contained 2% SDS and 1.25% β-mercaptoethanol.
2. The samples were then loaded and resolved on a on a Criterion™ TGX™ (Tris-Glycine eXtended) precast gel (4-20%) (see table above for amount protein per lane).
3. Proteins were transferred onto PVDF membrane by tank transfer and protein transfer was confirmed by using the ChemiDoc XRS imaging system.
4. The immunoblot membrane was blocked in Tris buffered saline (TBS) containing 0.05% Tween-20 (TTBs) and 3% non-fat dry milk powder (blocking buffer) for between 30-45 minutes at room temperature with gentle agitation on a rotary shaker at 100 rpms.
5. The membrane was then immersed with the protein side up in the primary antibody solution (for details see table above) diluted in TTBS containing 1% non-fat dry milk powder for 4 hours at room temperature with gentle agitation.
6. Following a one rinse and three washes for 5 minutes each at room temperature with TTBS, the membrane was incubated in the secondary antibody (for details see table above) diluted in TTBS containing 1% non-fat dry milk for 45 minutes at room temperature with gentle agitation.
7. The membrane was then rinsed once and washed twice with TTBS for 2 minutes and then 4 minutes respectively at room temperature. A final wash in TBS only at room temperature for 5 minutes was then performed.
8. After draining away excess TBS, the membrane was incubated for 2 minutes at room temperature with HRP substrate reagent (prepared just prior to use). Signals were detected using the ChemiDoc XRS imaging system.

## EXPERIMENTAL NOTES

EGFR P05, EGFR P29, EGFR P86 and EGFR M89 were immunoreactive with an epitope at around the expected MW in A549 lysate but not in the HeLa lysate (smear) or the Human lung tissue lysate. EGFR P04 also had this pattern of immunoreactivity although the signal is very weak under these conditions. EGFR M04, EGFR P03, EGFR P06 and EGFR P07 demonstrated some weak immunoreactivity but this may be non-specific as the MWs are quite varied and not at the expected MW for EGFR. EGFR M03 and EGFR M05 did not exhibit immunoreactivity in any of the tested lysates under the experimental conditions.

## 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 $307, €267, £198.

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

Images of Superstar EGFR antibodies: