

# **CERTIFICATE OF ANALYSIS**

| Important Note:           | Centrifuge before opening to   | entrifuge before opening to ensure complete recovery of vial contents. |                  |  |  |
|---------------------------|--|--|------------------|--|--|
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| :                         | K67123M  | Lot #:   | 11L34612         |  |  |
| Description:              | MAb to EGFR pTyr 845<br>Monoclonal Antibody to Human Epidermal Growth Factor Receptor (EGFR), Phosphorylated at<br>Tyrosine 845  |  |                  |  |  |
| Specificity:              | Binding of EGF leads to autophosphorylation of the EGF Receptor at 5 tyrosine residues. In addition, EGF Receptor is phosphorylated by members of the src kinase family at different tyrosine residues, phosphotyrosine 845 in the activation loop being the major site (tyrosine 845 is not an autophosphorylation site). EGFR acquires its full kinase activity only upon autophosphorylation <u>and</u> phosphorylation at tyrosine 845. Clone 12A3 allows the detection of EGFR activation after interaction with src kinases. Specifically recognizes EGFR phosphorylated at Tyrosine 845. The antibody does not interact with the non-phosphorylated EGFR nor with unrelated Tyrosine-phosphorylated proteins. Reacts with Human, mouse and rat. |  |                  |  |  |
| Clone:                    | 12A3   |  |                  |  |  |
| Host Animal:              | Mouse  | Isotype:   | IgG <sub>1</sub> |  |  |
| Source:                   | Cell Culture   |  |                  |  |  |
| Immunogen:                | Phosphopeptide Conjugate   |  |                  |  |  |
| Format:                   | Purified, Lyophilized<br>Reconstitute with 1 mL water for 15 r   | minutes at room temperature.   |                  |  |  |
| Purification:             | Thiophilic adsorption and size exclusion chromatography.   |  |                  |  |  |
| Concentration:            | 100 µg/mL (prior to lyophilization).   |  |                  |  |  |
| Buffer:                   | Lyophilized from 1 mL PBS, PEG an  | d Sucrose.   |                  |  |  |
| Preservative:             | 0.09% Sodium Azide (prior to lyophi  | lization).   |                  |  |  |

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## **Applications:**

Immunoblotting: 0.1 µg/mL for HRP/ECL detection. Recommended Blocking Buffer CPPT: 0.5% (w/v) casein, 1% (w/v) PEG 4000, 1% (w/v) Polyvinylpyrrolidone (PVP), 0.1% Tween 20, 10 mM Tris/HCl, pH 7.4, 150 mM Sodium Chloride.

|                              | co | HepG2<br>EGF VH | co | A 549<br>EGFVH |
|------------------------------|----|-----------------|----|----------------|
| 200 <b>—</b><br>116 <b>—</b> | ł  |                 |    |                |
| 66 <b></b><br>45 <b></b>     |    |                 |    |                |
| 31 —                         |    |                 |    |                |

#### Phosphospecificity

Whole cell extracts of control (co), EGF stimulated (EGF) or pervanadate treated (VH) HepG2 and A549 tumor cells were applied to SDS-PAGE (20,000 cells per lane) and transferred to a PVDF membrane. The blot was probed with  $0.5 \mu g/ml \ K67123M$  for 1 hour at RT and developed by ECL (exp. time: 30 sec).

#### ELISA: 0.1 µg/mL

Each laboratory should determine an optimum working titer for use in its particular application. Other applications have not been tested but use in such assays should not necessarily be excluded.

- **Storage:** Store lyophilized product at -20°C. After reconstitution, aliquot and store at -80°C. Thawed aliquots may be stored at 2–8°C for up to 3 months.
- Warning: This product contains sodium azide, which has been classified as Xn (Harmful) in European Directive 67/548/EEC in the concentration range of 0.1–1.0%. When disposing of this reagent through lead or copper plumbing, flush with copious volumes of water to prevent azide build-up in drains.

## **Includes Positive Control:**

| Description:  | Cell lysate of 2 x $10^6$ EGF treated HepG2 cells.  |
|---------------|---|
| Format:       | Lysate, Lyophilized<br>Reconstitute with 200 µL water. After complete solubilization of the proteins, add 200 µL 2x SDS-PAGE<br>sample buffer and incubate at 90°C for 5 minutes. |
| Applications: | For Western Blot applications: 20 µL/lane (mini gel) for HRP/ECL detection.   |
| Storage:      | Store lyophilized product at -20° C. After reconstitution, aliquot and store at -20°C. Avoid multiple freeze/thaw cycles.   |

lobut Ott

Signature

04 Dec 2015

Date

FOR RESEARCH OR FURTHER MANUFACTURING USE ONLY