

CERTIFICATE OF ANALYSIS

| Important Note: | Centrifuge before opening to ensure complete recovery of vial contents. | | | | | |
|-----------------------|---|----------|------------------|--|--|--|
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| Catalog #: | K67112M | Lot #: | 5H22619 | | | |
| Description: | MAb to EGFR, pTyr 1045 Monoclonal Antibody to Human Epidermal Growth Factor Receptor (EGFR), phosphorylated at Tyrosine 1045. | | | | | |
| Specificity: | Recognizes EGFR phosphorylated at Tyrosine 1045. Epitope: Phosphotyrosine pY1045 L Q R pY S S D. Reacts with Human and mouse. | | | | | |
| Host Animal: | Mouse | Isotype: | IgG ₁ | | | |
| Source: | Cell Culture | | | | | |
| Immunogen: | Phosphopeptide conjugated to KLH. | | | | | |
| Format: | Purified, Liquid | | | | | |
| Purification: | Thiophilic adsorption and size exclusion chromatography. | | | | | |
| Concentration: | 0.1 mg/mL | | | | | |
| Buffer: | Phosphate Buffered Saline, PEG, Sucrose containing 50% Glycerol. | | | | | |
| Preservative: | 0.09% Sodium Azide | | | | | |



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Applications:

Immunoblotting: 0.5 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.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | _ |
|------------------------------|---|---|---|---|---|---|---|---|---|
| 200 — 116 — | - | - | - | - | - | - | - | - | |
| 66 — 45 — | | | | | | | | | |
| 31— | | | | | | | | | 4 |
| 19242 | | | | | | | | | |

EGFR Activation

Serum starved MDA-MB 468 cells were incubated with 10 ng/mL EGF for the indicated times. Whole cell lysates were prepared and separated by SDS-PAGE (20,000 cells/lane). The blot was probed with 0.5 µg/mL K67112M for 1 hour at RT and developed by ECL (exp. time: 30 sec).

| Lane 1: Control | Lane 5: 1 hour EGF |
|--------------------|--------------------|
| Lane 2: 5 min EGF | Lane 6: 2 hour EGF |
| Lane 3: 15 min EGF | Lane 7: 4 hour EGF |
| Lane 4: 30 min EGF | Lane 8: 8 hour EGF |

Immunoprecipitation: Use at 1-10 µg per 10⁶ pervanadate-treated A431 cells. ELISA: 0.05 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:** Upon receipt, aliquot and store (up to 1 year) at -20°C. Avoid multiple freeze/thaw cycles. Safety Notes (s): Refer to the appropriate Safety Data Sheet (SDS) for additional information.

Includes Positive Control:

Description: Cell lysate of pervanadate treated HepG2 cells. Format: Lysate, Lyophilized Reconstitute with 200 µL water. After complete solubilization of the proteins, add 200 µL SDS-PAGE sample buffer and incubate at 90°C for 5 minutes. **Applications:** For Western Blot applications: 20 µL/lane (mini gel) for HRPO/ECL detection. 20µL is approximately 20,000 to 80,000 cells. Note: Lyophilized cell lysate contains SDS and is not recommended for applications with native proteins such as Immunoprecipitation. **Storage:** Store lyophilized product at -20°C. After reconstitution, aliquot and store at -20°C. Avoid multiple freeze/thaw cycles.

Brenda Dum

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