Anti-Human EGFR Antibody [PSH19-10] - BSA and Azide free (Detector)

HA724044

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	ELISA(Det)
Clone number:	PSH19-10

Description: Epidermal growth factor receptor (EGFR) is a transmembrane protein that is activated by binding of its specific ligands, including epidermal growth factor and transforming growth factor alpha (TGF-α). ErbB2 has no known direct activating ligand, and may be in an activated state constitutively or become active upon heterodimerization with other family members such as EGFR. Upon activation by its growth factor ligands, EGFR undergoes a transition from an inactive monomeric form to an active homodimer. – although there is some evidence that preformed inactive dimers may also exist before ligand binding. In addition to forming homodimers after ligand binding, EGFR may pair with another member of the ErbB receptor family, such as ErbB2/Her2/neu, to create an activated heterodimer. There is also evidence to suggest that clusters of activated EGFRs form, although it remains unclear whether this clustering is important for activation itself or occurs subsequent to activation of individual dimers.

Immunogen: Recombinant protein within Human EGFR aa 25-645 (HA211104).

Positive control: Recombinant Human EGFR protein (HA211104).

Subcellular location: Cell membrane, Endoplasmic reticulum membrane, Golgi apparatus membrane, Nucleus membrane, Endosome, Endosome membrane, Nucleus; Secreted.

Database links: SwissProt: P00533 Human

Recommended Dilutions:

ELISA(Det) Use at an assay dependent concentration. Can be paired for Sandwich ELISA with Rabbit monoclonal [PSH19-09] to Human EGFR antibody (Capture) (HA724043) and Recombinant Human EGFR protein (HA211104) as the standard. The reference range value is 78.1-20,000 pg/mL.

Storage Buffer: 1*PBS (pH7.4).

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

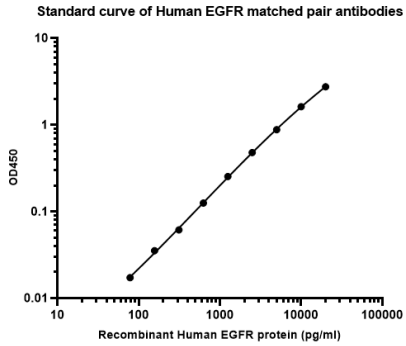

Fig1: Sandwich ELISA analysis of Human EGFR matched pair antibodies

Capture: HA724043, Human EGFR Rabbit mAb [PSH19-09]
Detector: HA724044, Human EGFR Rabbit mAb [PSH19-10]

Elisa assay was performed by coating wells of a 96-well plate with 100 µl per well of capture antibody (HA724043) diluted in carbonate/bicarbonate buffer, at a concentration of 2 µg/mL overnight at 4℃. Wells of the plate were washed, blocked with 150 µl 0.05% tween-20 1% BSA blocking buffer, and incubated with serial diluted Recombinant Human EGFR protein (HA211104) starting from 20,000 pg/ml to 0 pg/ml and detect antibody (HA724044, Biotin, 0.2 µg/ml) for 1 hour at 30℃ with shaking. Then the plate was washed and incubated with 100 µl per well of SA-HRP for 0.5 hour at 30℃ with shaking. Detection was performed using an Ultra TMB Substrate for 10 minutes at room temperature in the dark. The reaction was stopped with sulfuric acid and absorbances were read on a spectrophotometer at 450 nm.

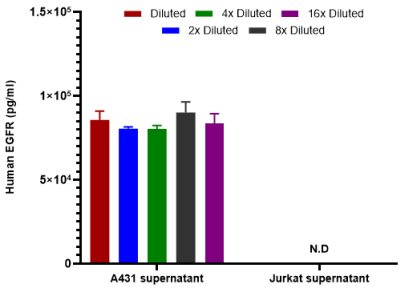

Fig2: Interpolated concentrations of native EGFR in A431 and Jurkat cell
culture supernatant.

Capture: HA724043, Human EGFR Rabbit mAb [PSH19-09]
Detector: HA724044, Human EGFR Rabbit mAb [PSH19-10]

Interpolated concentration of native EGFR was measured in duplicate at different sample concentrations and interpolated from the EGFR standard curves. The interpolated dilution factor corrected values were plotted (mean +/- SD, n=2). The mean EGFR concentration was determined to be 84,020 pg/ml in A431 cell culture supernatant and undetectable in Jurkat cell culture supernatant.

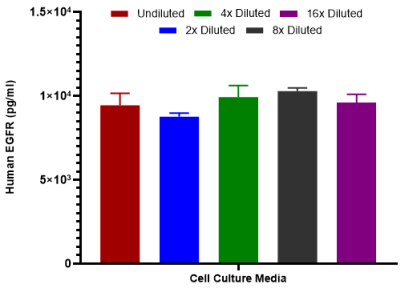

Fig3: Interpolated concentrations of spiked EGFR in cell culture media samples.

Capture: HA724043, Human EGFR Rabbit mAb [PSH19-09]
Detector: HA724044, Human EGFR Rabbit mAb [PSH19-10]

The concentrations of EGFR were measured in duplicates, interpolated from the EGFR standard curves and corrected for sample dilution. diluted samples are as follows: 50% cell culture media with FBS. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2).

Background References

1. Levantini E et al. EGFR signaling pathway as therapeutic target in human cancers. Semin Cancer Biol. 2022 Oct
2. Schramm F et al. EGFR Signaling in Lung Fibrosis. Cells. 2022 Mar