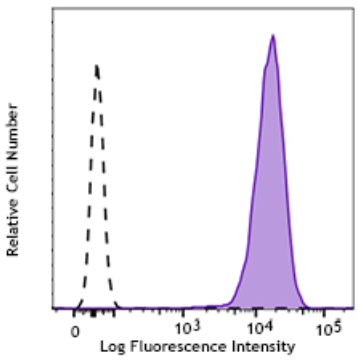
SONY

Product Data Sheet

Alexa Fluor® 647 anti-EGFR

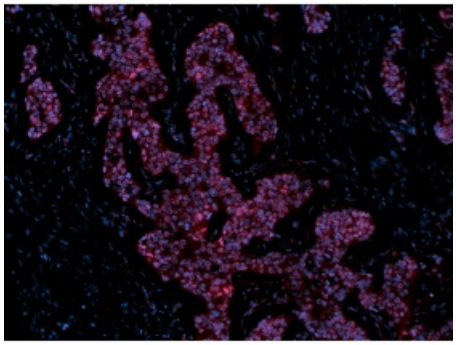
Catalog # / Size:	5269520 / 100 tests 5269515 / 25 tests
Clone:	A19002A
Isotype:	Mouse IgG1, κ
Immunogen:	Synthetic peptide from human EGFR
Reactivity:	Human
Preparation:	The antibody was purified by affinity chromatography and conjugated with Alexa Fluor® 647 under optimal conditions.
Formulation:	Phosphate-buffered solution, pH 7.2, containing 0.09% sodium azide and 0.2% (w/v) BSA (origin USA)
Workshop Number:	IV 103
Concentration:	Lot-specific



A-431 cells were stained intracellularly with anti-EGFR (clone A19002A) Alexa Fluor® 647 (filled histogram) or mouse IgG1, κ isotype control (open histogram).

Applications:

Applications:	Immunohistochemistry, Intracellular Staining for Flow Cytometry
Recommended Usage:	Each lot of this antibody is quality control tested by intracellular immunofluorescent staining with flow cytometric analysis. For flow cytometric staining, the suggested use of this reagent is 5 µL per million cells in 100 µL staining volume or 5 µL per 100 µL of whole blood. It is recommended that the reagent be titrated for optimal performance for each application. * Alexa Fluor® 647 has a maximum emission of 668 nm when it is excited at 633 nm / 635 nm.
Application Notes:	This clone was tested for ICC using 4% PFA-fixed A431 cells permabilized with methanol or Triton X-100. While both permeabilization methods were compatible with the antibody, methanol enabled superior staining. This clone is predicted to recognize isoform I based off of complete sequence homology of the immunizing peptide and the corresponding region of the isoform.



Formalin-fixed paraffin-embedded human breast cancer tissue slices were deparaffinized and rehydrated. Antigen retrieval was done with Tris-Buffered Saline 1X (1.0 M, pH 7.4) at 95°C for 40 minutes, washed with PBS/0.05% Tween 20 twice for five minutes, permeabilized with 0.5% Triton X-100 for ten minutes, and blocked with 5% FBS and 0.2% gelatin for 30 minutes. Then, the slices were stained with 5 µg/mL anti-EGFR (clone A19002A) Alexa Fluor® 647 (red) at 4°C overnight. Nuclei were counterstained with DAPI (green). The image was captured with a 10X objective.

Application References:

1. Kubagawa H, *et al*. 2009. *J Exp Med*. 206(12): 2779-93.
2. Kubagawa H, *et al*. 2014. *Monoclon Antib Immunodiagn Immunother.* 33(6): 393– 400.

Description: Epidermal Growth Factor Receptor (EGFR) is a receptor tyrosine kinase that links extracellular mitogenic ligand binding to complex downstream signaling cascades. Initial ligand binding results in receptor oligomerization and autophosphorylation of multiple tyrosine residues within cytosolic domains of the the protein. These phosphorylation events stabilize the EGFR kinase activation loop and lead to the recruitment of adaptor proteins and other downstream effectors. EGFR stimulation leads to activation of RAS-RAF-MEK-ERK, PI3 kinase-AKT, PLCγ-PKC and STAT signaling cascades, and constitutive activation of the receptor promotes tumorigenesis in multiple cancers.

Antigen References:

1. Hunter T and Cooper JA. 1981. *Cell*. 24:741.
2. Gill GN and Lazar CS. 1981. *Nature.* 293:305.
3. Reynolds FH, *et al.* 1981. *Nature.* 292:259
4. Zhou M, *et al.* 2013. *Cancer Res.* 23:7056-67