

RAF1 | Validation File

TARGET mouse RAF1

CLONE NAME EMI411E

DESCRIPTION rat monoclonal

ANTIGEN USED His-RAF1 (fragment 189-353)

ISOTYPE IgG2a

SPECIES REACTIVITY mouse and human

LOCALIZATION membrane and cytoplasm

POSITIVE CONTROL A549 cell line

STORAGE BUFFER Tissue culture supernatant: 0.02% sodium azide

Purified antibody: PBS plus 1%BSA and 0.02% sodium azide. MAb concentration: 1mg/ml

STORAGE Aliquot and store at 4C. Do not freeze

 Recommended

 Inconclusive

 Not Recommended

 Not Tested

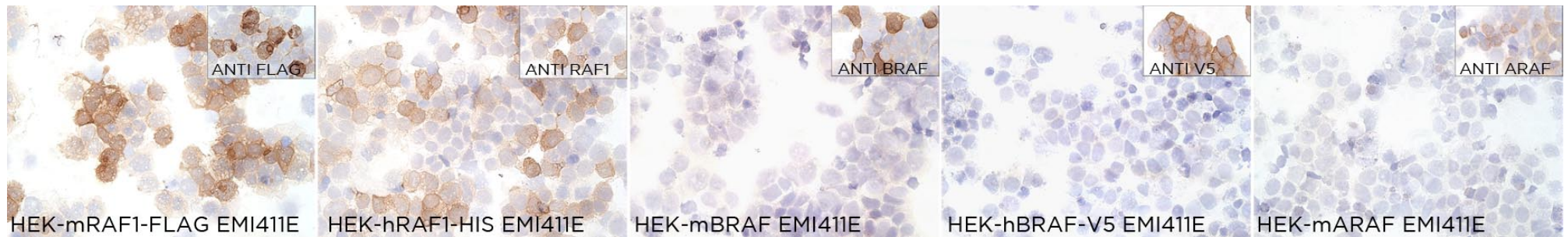
APPLICATIONS

● | ICC | Immunocytochemistry

EMI411E mAb is able to detect human and mouse RAF1 protein in immunocytochemistry

DILUTION no dilution (neat supernatant)

To confirm that EMI411E mAb recognizes human and mouse RAF1 protein and do not cross react with mA-RAF and m/hBRAF, immunocytochemistry on frozen cytospin preparations of human and mouse proteins expressed in HEK293T were performed. Anti-FLAG, RAF1, B-RAF and V5 Abs were used as positive controls.



● | WB | Western Blotting

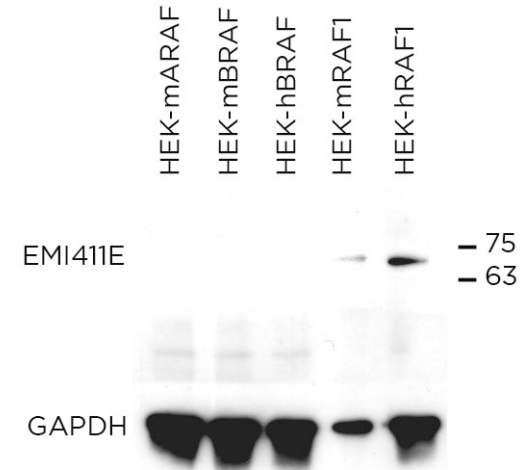
EMI411E mAb is able to detect mouse and human RAF1 protein by WB.

DILUTION no dilution (neat supernatant)
1:500 purified antibody

LANES

Lane 1 HEK-mARAF (10ug) (-)
Lane 2 HEK-mBRAF (10ug) (-)
Lane 3 HEK-hBRAF (10ug) (-)
Lane 4 HEK-mRAF1 (10ug) (+)
Lane 5 HEK-hRAF1 (10ug) (+)

GAPDH was used as loading control



LANES

Lane 1 mouse Lung Adenocarcinoma RAF1 KO (20ug) (-)
Lane 2 mouse Lung Adenocarcinoma RAF1 WT (20ug) (+)
Lane 3 human A549 (RAF1 WT) (20ug) (+)
Lane 4 human PULM24 (RAF1 WT) (20ug) (+)

Predicted molecular weight: **73kDa**
Observed molecular weight: **73kDa**



● | IHC-P-M | **Immunohistochemistry (paraffin)**

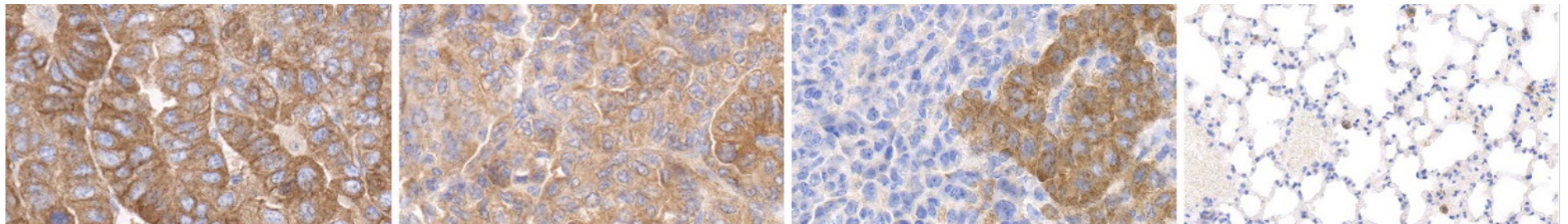
EMI411E mAb can be used to detect RAF1 protein in mouse paraffin tissues

TISSUE SAMPLE mouse lung adenocarcinoma and normal mouse lung

DILUTION no dilution (neat supernatant)
1:200 purified antibody

ANT. RETRIEVAL High pH9

DETECTION SYSTEM Autostainer Link® (Dako, Agilent). Rabbit anti-rat Vector + Novolink Polymer



RAF1 expressing murine $Kras^{G12V}/p53^{KO}$ lung adenocarcinoma

Partial genetic deletion of RAF1 expression in murine $Kras^{G12V}/p53^{KO}$ lung adenocarcinoma

Normal lung tissue showing expression of RAF1 in the resident macrophages.

● | IF | **Immunofluorescence (paraffin)** Not done

● | IP | **Immunoprecipitation** Not done

● | IHC-F | **Immunohistochemistry (frozen)** Not done