mNGFR | ***Validation File***

TARGET NGFR Tumor necrosis factor receptor superfamily member 16
CLONE NAME NORI146C
DESCRIPTION rat monoclonal
ANTIGEN USED His-mNGFR (276-427aa) protein
ISOTYPE IgG2a
SPECIES REACTIVITY mouse
LOCALIZATION transmembrane/cytoplasm
POSITIVE CONTROL mouse liver
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

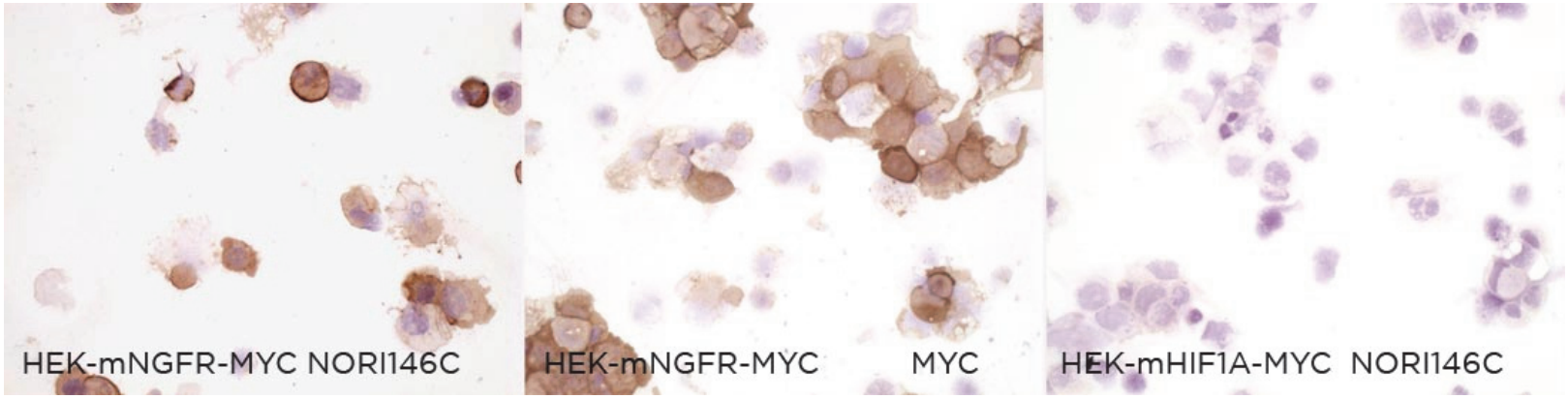
Recommended Inconclusive Not Recommended Not Tested

APPLICATIONS

| ICC | ***Immunocytochemistry***

NORI146C mAb is able to detect mouse NGFR protein in immunocytochemistry.

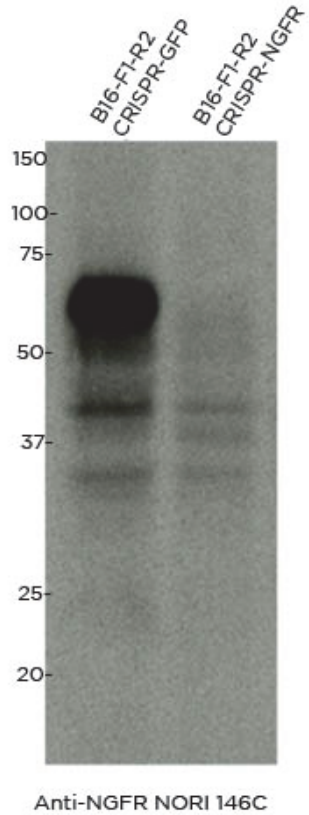
To confirm that NORI146C mAb recognizes mouse NGFR protein, immunocytochemistry on frozen cytospins preparations of MYC-tagged mNGFR expressed in HEK293 was performed. Anti-MYC was used as positive control. Cytospin of HEK293 transfected with mouse HIF1a were used as a negative control.



| WB | ***Western Blotting***

NORI146C mAb is able to detect mouse NGFR protein by WB.

DILUTION no dilution (neat supernatant)



Anti-NGFR NORI 146C

Predicted molecular weight: ***45,6KDa***
Observed molecular weight: ***60KDa***

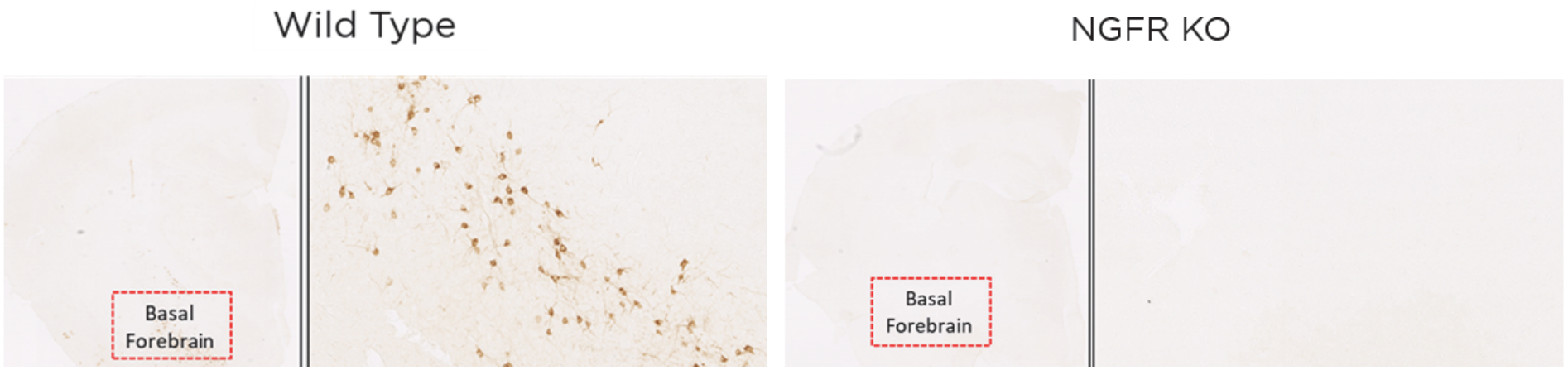
LANES

Lane 1 Melanoma Cell line B16-F1-R2 WT (30ug) (+)
Lane 2 Melanoma Cell line B16-F1-R2 KO (30ug) (+)

| IHC-F | ***Immunohistochemistry (frozen)***

NORI146C mAb is able to detect mouse NGFR protein by immunohistochemistry in cryopreserved samples. NGFR KO mouse model B6.129S4-Ngfrtm1Jae/6J.OlaHsd was used as control.

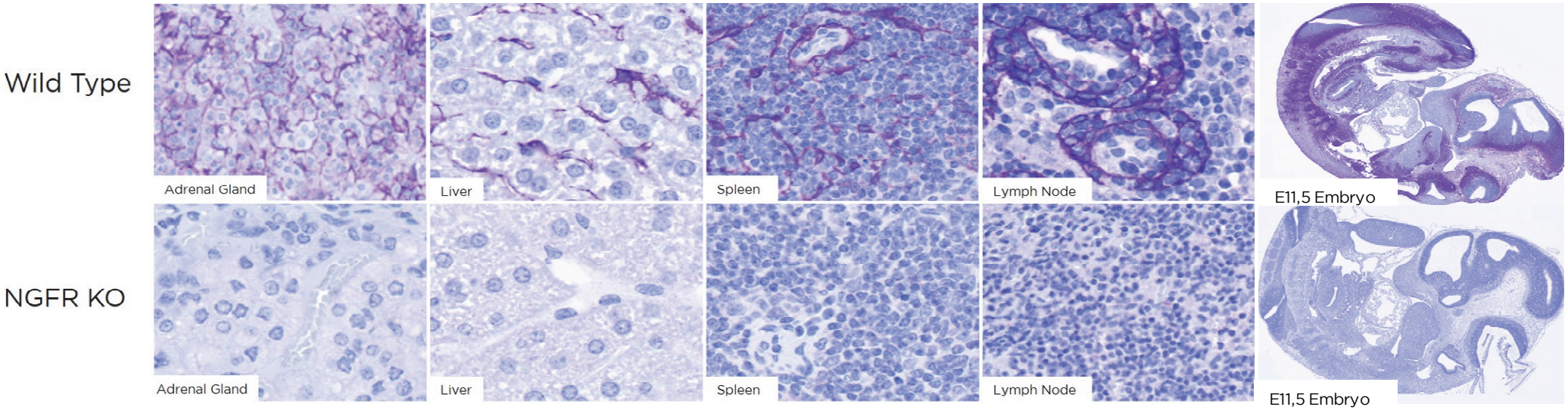
TISSUE SAMPLE Brain
DILUTION 1:200 (supernatant)
DETECTION SYSTEM Floating immunohistochemistry in cryopreserved samples



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

NORI146C mAb is able to detect mouse NGFR protein by immunohistochemistry in paraffin samples of NGFR KO mouse model B6.129S4-Ngfrtm1Jae/6J.OlaHsd

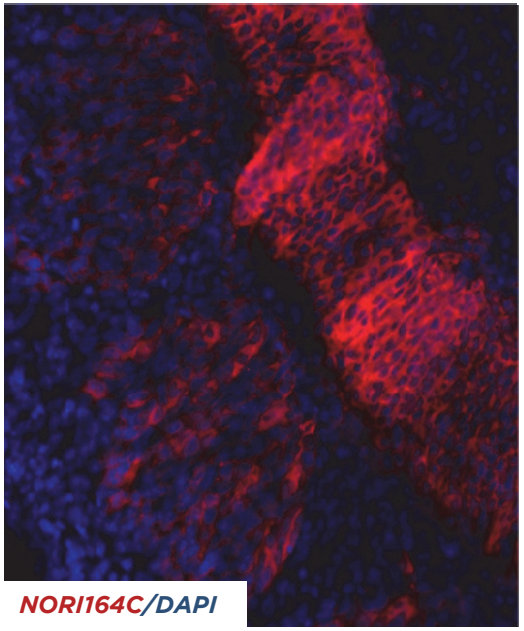
TISSUE SAMPLE Adrenal gland, liver, spleen, lymph node and embryo
DILUTION 1:10 (supernatant)
ANTIGEN RETRIEVAL High pH
DETECTION SYSTEM Omnimap anti rat + Anti-Rabbit HRP



| IF | *Immunofluorescence (frozen tissue)*

NORI146C mAb is able to detect mouse NGFR protein by immunofluorescence in OCT embedded tissue samples.

TISSUE SAMPLE Dorsal root ganglions of C57BL/6J.OlaHsd E10.5 Mouse embryo
SECONDARY ANTIBODY Goat anti-Rat AF555 (Thermo 1/1000)
DILUTION 1:1 (supernatant)
1:500 purified antibody (1mg/ml)
ANTIGEN RETRIEVAL Immunofluorescence in OCT embedded samples



NORI164C/DAPI

| IP | ***Immunoprecipitation***

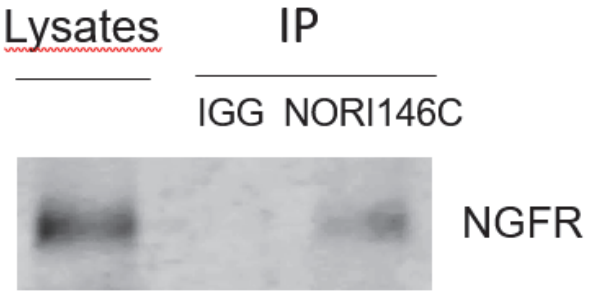
NORI146C mAb is able to immunoprecipitate endogenous mouse NGFR protein from murine B16F1R2 cell-derived exosomes.

0,5mg of B16F1R2 cell derived exosomes were lysed in 1ml of TNTE buffer (20nM TRIS HCL ph7,5; 150nM NaCL; 5mM EDTA; 0,5% TRITON 100%; 10% Glicerol)+phosphatase and protease inhibitor cocktail 1h at 4ºC in a rotor. Lysates were centrifuged 15min at 15000rpm and supernatants were incubated with anti-IGG isotype control (abcam18450) or NORI146C mAB (2ug/mg of total protein; from purified antibody) with 1mg/ml BSA overnight in a rotor at 4ºC. The following day, Protein G sepharose (20ul) were added and incubated for 3-4h at 4ºC. 4-6 washes at 2000rpm 2min were performed and IP was loaded in a 10% acrilamide/bisacrilamide gel. Western blot was developed using NORI138B antibody (1:1000) as indicated before

Predicted molecular weight: ***45kDa***
Observed molecular weight: ***75kDa***

LANES

Lane 1. Lysates from B16F1R2 derived exosomes (10ug)
Lane 2. B16F1R2 derived exosomes IP anti-IGG
Lane 2 B16F1R2 derived exosomes IP NGFR



| FC | ***Flow Cytometry*** Not suitable