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Anti-NIRF Antibody Picoband™ (monoclonal, 6B5)

Catalog # ABO15076

Product Information

Application	WB, IF, ICC, FC
Primary Accession	Q96PU4
Host	Mouse
Isotype	Mouse IgG2b
Reactivity	Rat, Human
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-NIRF Antibody Picoband™ (monoclonal, 6B5) . Tested in Flow Cytometry, IF, ICC, WB applications. This antibody reacts with Human, Rat.
Reconstitution	Adding 0.2 ml of distilled water will yield a concentration of 500 □g/ml.

Additional Information

Gene ID	115426
Other Names	E3 ubiquitin-protein ligase UHRF2, 2.3.2.27, Np95/ICBP90-like RING finger protein, Np95-like RING finger protein, Nuclear protein 97, Nuclear zinc finger protein Np97, RING finger protein 107, RING-type E3 ubiquitin transferase UHRF2, Ubiquitin-like PHD and RING finger domain-containing protein 2, Ubiquitin-like-containing PHD and RING finger domains protein 2, UHRF2, NIRF, RNF107
Calculated MW	89985
Application Details	Western blot, 0.25-0.5 □g/ml, Human Immunocytochemistry/Immunofluorescence, 5 □g/ml, Human Flow Cytometry, 1-3 □g/1x10^6 cells, Human, Rat
Source	Eukaryota
Contents	Each vial contains 4 mg Trehalose, 0.9 mg NaCl and 0.2 mg Na2HPO4.
Clone Names	Clone: 6B5
Immunogen	A synthetic peptide corresponding to a sequence at the N-terminus of human NIRF, identical to the related mouse and rat sequences.
Purification	Immunogen affinity purified.
Storage	At -20°C for one year from date of receipt. After reconstitution, at 4°C for one month. It can also be aliquotted and stored frozen at -20°C for six months. Avoid repeated freezing and thawing.

Protein Information

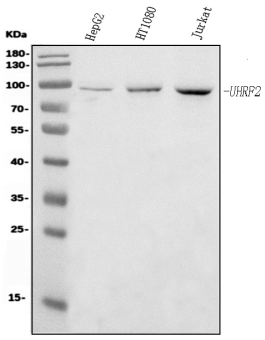
Name	UHRF2
Synonyms	NIRF, RNF107
Function	E3 ubiquitin ligase that plays important roles in DNA methylation, histone modifications, cell cycle and DNA repair (PubMed:15178429, PubMed:23404503, PubMed:27743347, PubMed:29506131). Acts as a specific reader for 5-hydroxymethylcytosine (5hmC) and thereby recruits various substrates to these sites to ubiquitinate them (PubMed:24813944, PubMed:27129234). This activity also allows the maintenance of 5mC levels at specific genomic loci and regulates neuron-related gene expression (By similarity). Participates in cell cycle regulation by ubiquitinating cyclins CCND1 and CCNE1 and thereby inducing G1 arrest (PubMed:15178429, PubMed:15361834, PubMed:21952639). Also ubiquitinates PCNP leading to its degradation by the proteasome (PubMed:12176013, PubMed:14741369). Plays an active role in DNA damage repair by ubiquitinating p21/CDKN1A leading to its proteasomal degradation (PubMed:29923055). Also promotes DNA repair by acting as an interstrand cross-links (ICLs) sensor. Mechanistically, cooperates with UHRF1 to ensure recruitment of FANCD2 to ICLs, leading to FANCD2 monoubiquitination and subsequent activation (PubMed:30335751). Contributes to UV-induced DNA damage response by physically interacting with ATR in response to irradiation, thereby promoting ATR activation (PubMed:33848395).
Cellular Location	Nucleus {ECO:0000255|PROSITE-ProRule:PRU00358, ECO:0000269|PubMed:12176013, ECO:0000269|PubMed:23404503, ECO:0000269|PubMed:27129234, ECO:0000269|PubMed:27743347, ECO:0000269|PubMed:29923055, ECO:0000269|PubMed:30335751}. Chromosome. Note=Enriched at genomic loci that are enriched for 5-hydroxymethylcytosine (5hmC)

Background

E3 ubiquitin-protein ligase UHRF2 is an enzyme that in humans is encoded by the UHRF2 gene. This gene encodes a nuclear protein which is involved in cell-cycle regulation. The encoded protein is a ubiquitin-ligase capable of ubiquinating PCNP (PEST-containing nuclear protein), and together they may play a role in tumorigenesis. The encoded protein contains an NIRF_N domain, a PHD finger, a set- and ring-associated (SRA) domain, and a RING finger domain and several of these domains have been shown to be essential for the regulation of cell proliferation. This protein may also have a role in intranuclear degradation of polyglutamine aggregates. Alternative splicing results in multiple transcript variants some of which are non-protein coding.

Images

Figure 1. Western blot analysis of NIRF using anti-NIRF antibody (M06294-1).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours.
The sample well of each lane was loaded with 30 ug of sample under reducing conditions.
Lane 1: human HepG2 whole cell lysates,
Lane 2: human HT1080 whole cell lysates,
Lane 3: human Jurkat whole cell lysates.



After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA for 50-90 minutes. Blocked the membrane with 5% non-fat milk/TBS for 1.5 hour at RT. The membrane was incubated with mouse anti-NIRF antigen affinity purified monoclonal antibody (Catalog # M06294-1) at 0.5 µg/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-mouse IgG-HRP secondary antibody at a dilution of 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1001) with Tanon 5200 system. A specific band was detected for NIRF at approximately 90 kDa. The expected band size for NIRF is at 90 kDa.

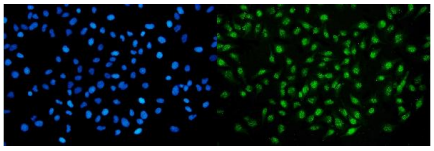


Figure 2. IF analysis of NIRF using anti-NIRF antibody (M06294-1).
NIRF was detected in an immunocytochemical section of Hela cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5 µg/mL mouse anti-NIRF Antibody (M06294-1) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Mouse IgG (BA1126) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

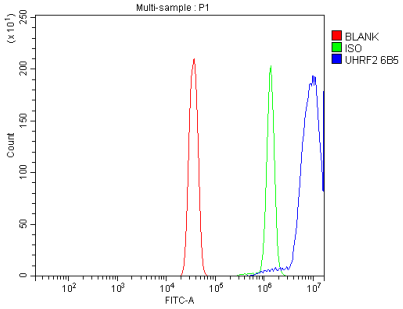


Figure 3. Flow Cytometry analysis of Hela cells using anti-NIRF antibody (M06294-1).
Overlay histogram showing Hela cells stained with M06294-1 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-NIRF Antibody (M06294-1, 1 µg/1x10^6 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 µg/1x10^6 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 µg/1x10^6) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

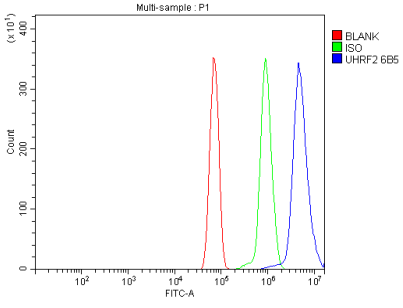


Figure 4. Flow Cytometry analysis of RH35 cells using anti-NIRF antibody (M06294-1).
Overlay histogram showing RH35 cells stained with M06294-1 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-NIRF Antibody (M06294-1, 1 µg/1x10^6 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 µg/1x10^6 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 µg/1x10^6) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.