Product Datasheet

TRF-2 Antibody (4A794.15) - BSA Free
NB100-56506

Unit Size: 0.1 mg

Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

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Reviews: 1 Publications: 116

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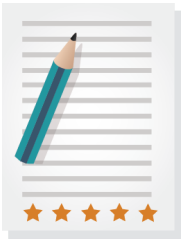
Updated 4/13/2025 v.20.1



NB100-56506

TRF-2 Antibody (4A794.15) - BSA Free

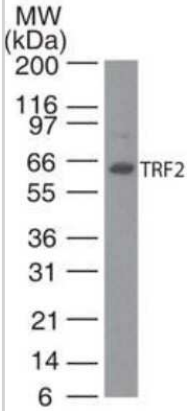
Product Information	
Unit Size	0.1 mg
Concentration	1 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	4A794.15
Preservative	0.02% Sodium Azide
Isotype	IgG1 Kappa
Purity	Protein G purified
Buffer	PBS
Target Molecular Weight	59.6 kDa

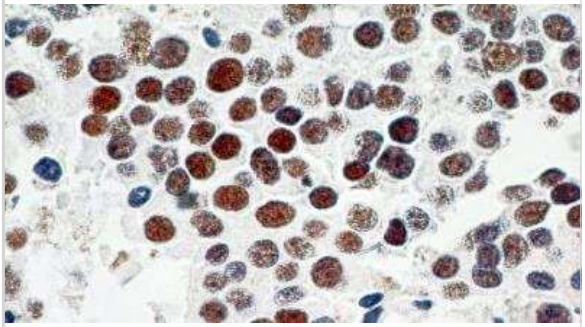
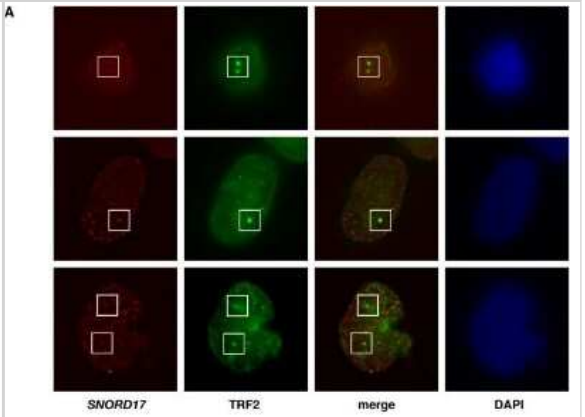
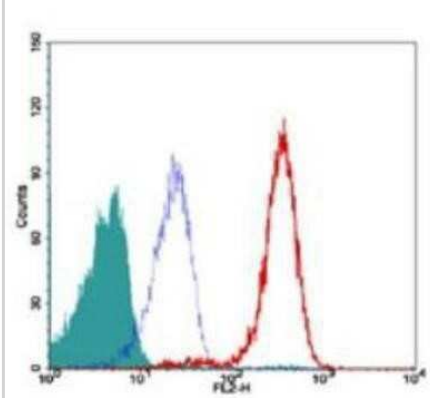
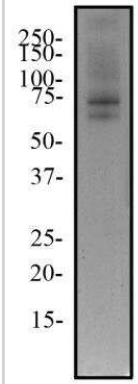
Product Description	
Description	The TRF2 antibody is referred to as both clone 4A794.15 and 4A794 in the published literature. The TRF2 antibody recognizes full-length TRF2 as well as TRF2 forms lacking both the N-terminal basic domain (B) and the telobox Myb-like C-terminal DNA-binding domain (M). TRF2 forms missing the B and M domains are often referred to as mutant TRF2. Although the exact epitope recognized by the TRF2 antibody has not been mapped, the scientific literature indicates it is in the D or L domain, but not in the B or M domain.
Host	Mouse
Gene ID	7014
Gene Symbol	TERF2
Species	Human, Mouse, Rat, Deer, Marsupial
Marker	Telomeres marker
Specificity/Sensitivity	The TRF2 antibody recognizes full-length TRF2 as well as TRF2 forms lacking both the N-terminal basic domain (B) and the telobox Myb-like C-terminal DNA-binding domain (M). TRF2 forms missing the B and M domains are often referred to as mutant TRF2. Although the exact epitope recognized by the TRF2 antibody has not been mapped, the scientific literature indicates it is in the D or L domain, but not in the B or M domain.
Immunogen	This TRF-2 Antibody (4A794.15) was developed against Baculovirus expressed whole length TRF2 protein, used for immunizing mice and splenocytes used to generate the hybridoma clone (NP_005643).

Product Application Details	
Applications	Western Blot, Simple Western, ELISA, Flow Cytometry, Flow (Intracellular), Immunoblotting, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin, Immunoprecipitation, Proximity Ligation Assay, Chromatin Immunoprecipitation (ChIP), CyTOF-ready

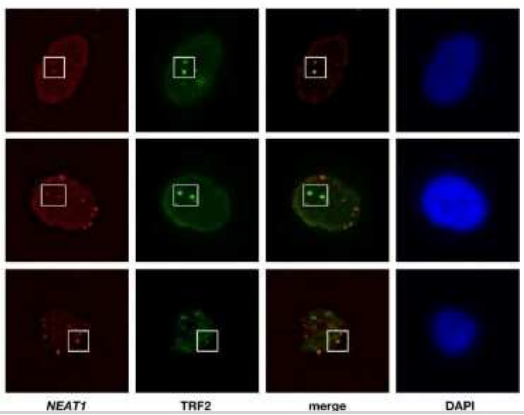
Recommended Dilutions	Western Blot 2-4 ug/ml, Simple Western 1:50, Flow Cytometry 0.1 ug/10^6 cells, ELISA 2 ug/ml, Immunohistochemistry 1:10 - 1:500, Immunocytochemistry/ Immunofluorescence 1:10 - 1:500, Immunoprecipitation 2 ug/10^6 cells, Immunohistochemistry-Paraffin 1:200, Immunohistochemistry-Frozen 5 ug/ml, Immunoblotting reported in scientific literature (PMID 23708666), Proximity Ligation Assay reported in scientific literature (PMID 27366950), Flow (Intracellular), Chromatin Immunoprecipitation (ChIP) 1:10-1:500, CyTOF-ready
Application Notes	TRF-2 may be detected as a single band or as a doublet in Western blot. Okabe (2000) described the doublet as 65 and 69 kDa using clone 4A794.15. Observed molecular weights could vary depending on molecular weight standards used and gel conditions. In Simple Western only 10 - 15 uL of the recommended dilution is used per data point. See Simple Western Antibody Database for Simple Western validation: Tested in HeLa lysate 1.0 mg/mL, separated by Size, antibody dilution of 1:50, apparent MW was 80 kDa. Separated by Size-Wes, Sally Sue/Peggy Sue. The observed molecular weight of the protein may vary from the listed predicted molecular weight due to post translational modifications, post translation cleavages, relative charges, and other experimental factors.

Images

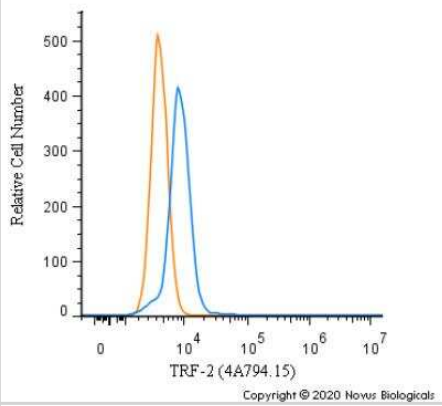
Simple Western: TRF-2 Antibody (4A794.15) [NB100-56506] - Image shows a specific band for TRF2 in 1.0 mg/mL of HeLa lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system. Non-specific interaction with the 230 kDa Simple Western standard may be seen with this antibody.	
Western Blot: TRF-2 Antibody (4A794.15) [NB100-56506] - Analysis in human Jurkat cell lysate at 2 ug/mL. Goat anti-mouse Ig HRP secondary antibody and PicoTect ECL substrate solution were used.	

Immunohistochemistry-Paraffin: TRF-2 Antibody (4A794.15) [NB100-56506] - Transitional cell carcinoma, urinary bladder, stained with TRF2 antibody (4 ug/mL), peroxidase-conjugate and DAB chromogen. Note specific nuclear staining. Tumor/normal adjacent tissue array slide was used for this test. Staining of formalin-fixed tissues is enhanced by boiling tissue sections in 10 mM sodium citrate buffer, pH 6.0 for 10-20 min followed by cooling at RT for 20 min.	
Immunocytochemistry/Immunofluorescence: TRF-2 Antibody (4A794.15) [NB100-56506] - Localization of candidate RNAs at telomeres. Localization of SNORD17 at telomeres in human U-2 OS cells. Cells were fixed and sequentially incubated with Abs against TRF-2 and AlexaFluor 488-conjugated anti-mouse IgG. Subsequently, the cells were hybridized with the RNA probes and subjected to fluorescence microscopy. Three different cells are shown. Image collected and cropped by CiteAb from the following publication (https://dx.plos.org/10.1371/journal.pone.0123387), licensed under a CC-BY license.	
Flow Cytometry: TRF-2 Antibody (4A794.15) [NB100-56506] - Intracellular flow cytometric analysis of TRF2 in 10^6 human Jurkat cells using 0.1 ug of NB100-56506. The shaded histogram represents cells alone, blue represents isotype control and red represents NB100-56506, anti-TRF2.	
Western Blot: TRF-2 Antibody (4A794.15) [NB100-56506] - Total protein from mouse 3T3 cells was separated on a 12% gel by SDS-PAGE, transferred to PVDF membrane and blocked in 5% non-fat milk in TBST. The membrane was probed with 2.0 ug/mL anti-TRF2 in 1% non-fat milk in TBST and detected with an anti-mouse HRP secondary antibody using chemiluminescence.	

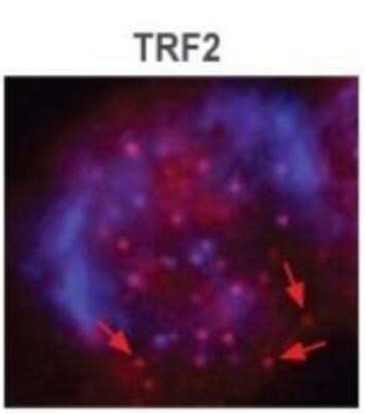
Immunocytochemistry/Immunofluorescence: TRF-2 Antibody (4A794.15) [NB100-56506] - Localization of candidate RNAs at telomeres. Localization of NEAT1 at telomeres in human U-2 OS cells. Cells were fixed and sequentially incubated with Abs against TRF-2 and AlexaFluor 488-conjugated anti-mouse IgG. Subsequently, the cells were hybridized with the RNA probes and subjected to fluorescence microscopy. Three different cells are shown. Image collected and cropped by CiteAb from the following publication (https://dx.plos.org/10.1371/journal.pone.0123387), licensed under a CC-BY license.



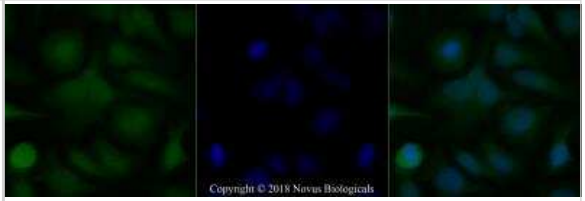
Flow Cytometry: TRF-2 Antibody (4A794.15) [NB100-56506] - An intracellular stain was performed on A431 cells with TRF2 Antibody [4A794.15] NB100-56506 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeabilized with 0.1% saponin. Cells were incubated in an antibody dilution of 1.0 ug/mL for 30 minutes at room temperature, followed by Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Dylight 550 (35503, Thermo Fisher).

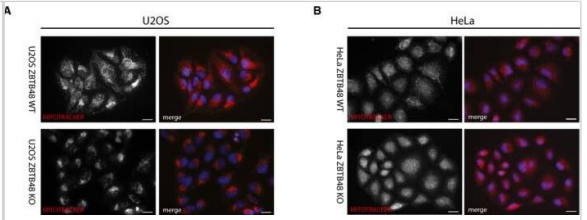
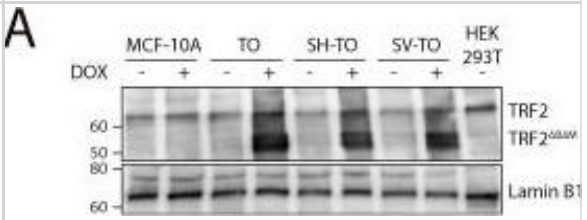


Immunocytochemistry/Immunofluorescence: TRF-2 Antibody (4A794.15) [NB100-56506] - Staining of TRF2-bound telomeres in human HeLa cells (Courtesy of Fotiadou, et al, 2004).

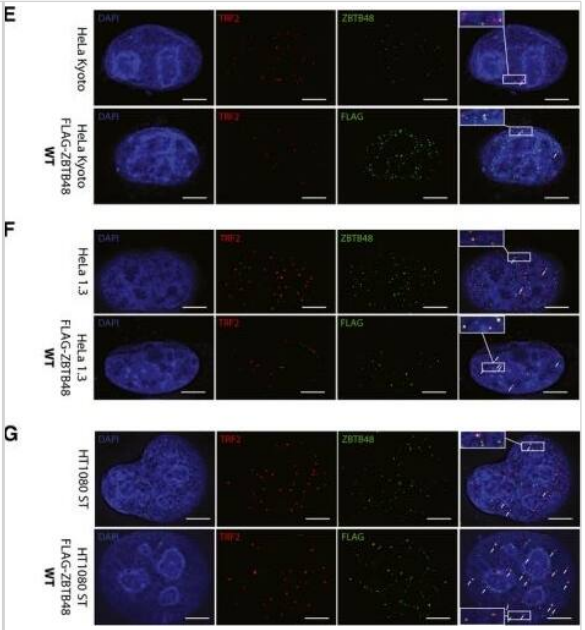


Immunocytochemistry/Immunofluorescence: TRF-2 Antibody (4A794.15) [NB100-56506] - HeLa cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X PBS + 0.5% Triton-X100. The cells were incubated with anti-TRF-2 (4A794.15) conjugated to FITC [NB100-56506F] at 5 ug/mL for 1 hour at room temperature. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.



Immunocytochemistry/ Immunofluorescence: TRF-2 Antibody (4A794.15) - BSA Free [NB100-56506] - ZBTB48□dependent loss of MTFP1 phenocopies MTFP1 depletionFluorescence microscopy analysis of the structure & localization of the mitochondrial network in HeLa WT & ZBTB48 KO clones. Mitochondria are marked with the MitoTracker dye (red), & nuclei are counterstained with DAPI (blue). Scale bars represent 20 µm.The same analysis as in (A) for U2OS WT & ZBTB48 KO clones. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/28500257), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	
Western Blot: TRF-2 Antibody (4A794.15) - BSA Free [NB100-56506] - TRF2ΔBΔM expression induces chromosome end-to-end fusions in all inducible cell lines(A) Immunoblots of MCF-10A, TO, SH-TO & SV-TO cell lines with & without DOX & HEK 293T. After 96 h of DOX treatment, the inducible cell lines expressed the truncated TRF2ΔBΔM protein (50 kDa); in contrast, uninduced cell lines, MCF-10A parental cell line & HEK 293T cells displayed only the endogen TRF2 protein (66 kDa). Lamin B1 was used as loading control. (B) After sustained expression of TRF2ΔBΔM for 96 h there was a significant increase in aberrant metaphases only in TO cells when compared to uninduced matched cells. (C) Nevertheless, the efficacy of the inducible system was validated by the statistical increase of cells with end-to-end fusions in all inducible cell lines. (D) Moreover, a high incidence of fusions per cell was found in all modified cell lines after sustained TRF2 depletion. Data was presented as mean + SEM. (E) Example of TO, SH-TO & SV-TO karyotypes after 96 h of TRF2ΔBΔM expression. Open arrows indicate clonal aberrations in the parental MCF-10A cell line. Insets in the karyotype show rearranged chromosomes stained with centromeric (green) & telomeric (red) PNA probes. Note the presence of telomere FISH signals at the fusion point of chromatid- or chromosome-type end-to-end fusions. Image collected & cropped by CiteAb from the following publication (https://www.oncotarget.com/lookup/doi/10.18632/oncotarget.25502), licensed under a CC-BY license. Not internally tested by Novus Biologicals.	

Immunocytochemistry/ Immunofluorescence: TRF-2 Antibody (4A794.15) - BSA Free [NB100-56506] - ZBTB48 ZnF11 necessary to bind to telomeres. Sequence□specific DNA pull□downs w/ either telomeric (TTAGGG)/a control sequence (GTGAGT) for FLAG□ZBTB48 WT, domain deletion constructs for different zinc finger & combinations of deletion constructs w/ ZnF10/11 point mutants. Domain structures indicated - right.Sequence□specific DNA pull□downs for FLAG□ZBTB48 WT & ZnF11 point mutant for telomeric repeat sequences of different phyla (green) & their respective scrambled controls (blue).Protein expression analysis of ZBTB48 by WB for cell lines used. GAPDH serves as a loading control.IF stainings for exogenous FLAG□ZBTB48 WT & point mutants for ZnF10 & ZnF11 in U2OS cells. The same analysis as in Fig 1E performed & average co□localization frequencies shown (n = 24–37 cells).Co□localization analysis of endogenous ZBTB48/exogenous FLAG□ZBTB48 WT w/ TRF2 in HeLa cells by IF (IF) staining. Image illustrating co□localization between ZBTB48/FLAG□ZBTB48 WT (green) & TRF2 (red) as a marker for telomeres is shown w/ DAPI (blue) used as nuclear counterstain. Co□localization events indicated by white arrows. Quantification of frequency of co□localization events (right) done after 3D reconstruction of acquired z□stacks (n = 30 cells).Co□localization analysis of endogenous ZBTB48/exogenous FLAG□ZBTB48 WT w/ TRF2 in HeLa 1.3 cells by IF (IF) staining analogous to (E) (n = 30 cells).Co□localization analysis of endogenous ZBTB48/exogenous FLAG□ZBTB48 WT w/ TRF2 in HT1080 super□telomerase cells by IF (IF) staining analogous to (E) (n = 30 cells).Data information: (D–G) Scale bars = 5 μm. Error bars indicate standard deviations, & P□values based on Student's t□test.Source data available online for this figure. Image collected & cropped by CiteAb from following publication (https://pubmed.ncbi.nlm.nih.gov/28500257), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Zhang L, Geng X, Wang F et al. 53BP1 regulates heterochromatin through liquid phase separation Nature communications 2022-01-18 [PMID: 35042897]

Yajun Wang, Wei Zhu, Yumi Jang, Joshua A Sommers, Gong Yi, Chandrakala Puligilla, Deborah L Croteau, Yibin Yang, Mihoko Kai, Yie Liu The RNA-binding motif protein 14 regulates telomere integrity at the interface of TERRA and telomeric R-loops Nucleic Acids Research 2023-12-11 [PMID: 37930826]

Zhang J, Zhang F, Porter KI et al. Telomere dysfunction in Tert knockout mice delays BrafV600E -induced melanoma development International journal of cancer 2023-09-20 [PMID: 37727982] (IHC-P, Mouse)

Details:
Dilution: 1:300

Hou J, Yun Y, Jeon B et al. Ginsenoside F1-Mediated Telomere Preservation Delays Cellular Senescence Int J Mol Sci 2023-09-19 [PMID: 37762556]

Jacome Burbano MS, Robin JD, Bauwens S et al. Non-canonical telomere protection role of FOXO3a of human skeletal muscle cells regulated by the TRF2-redox axis Communications biology 2023-05-25 [PMID: 37231173]

Details:
Dilution:1:200

Kliszczak M, Moralli D, Jankowska JD et al. Loss of FAM111B protease mutated in hereditary fibrosing poikiloderma negatively regulates telomere length Frontiers in cell and developmental biology 2023-06-05 [PMID: 37342232] (ICC/IF, Human)

Kliszczak M, Moralli D, Jankowska J et al. Loss of FAM111B protease mutated in hereditary fibrosing poikiloderma syndrome negatively regulates telomere length bioRxiv 2023-01-23

Dinami R, Pompili L, Petti E et al. MiR-182-3p targets TRF2 and impairs tumor growth of triple-negative breast cancer EMBO molecular medicine 2022-11-25 [PMID: 36426578] (IHC-P, Human)

Athmane N Comparing methods for visualising genomic loci in live mammalian cells Anal Chem 2017-11-10 [PMID: 29120617]

Smith S Persistent telomere cohesion protects aged cells from premature senescence Nat Commun 2020-07-05 [PMID: 32620872]

Mendez-Bermudez A, Lototska L, Pousse M et al. Selective pericentromeric heterochromatin dismantling caused by TP53 activation during senescence Nucleic acids research 2022-07-22 [PMID: 35819196] (ICC, Human)

Eguchi A, Torres-Bigio SI, Koleckar K, Birnbaum F TRF2 rescues pathogenic phenotypes in Duchenne muscular dystrophy cardiomyocytes derived from human iPSCs bioRxiv 2022-01-01 [PMID: 36719921] (WB)

More publications at http://www.novusbio.com/NB100-56506

Procedures

Immunocytochemistry/ Immunofluorescence Protocol for TRF-2 Antibody (NB100-56506)
Immunocytochemistry Protocol

Culture cells to appropriate density in 35 mm culture dishes or 6-well plates.

1. Remove culture medium and wash the cells briefly in PBS. Add 10% formalin to the dish and fix at room temperature for 10 minutes.
2. Remove the formalin and wash the cells in PBS.
3. Permeablize the cells with 0.1% Triton X100 or other suitable detergent for 10 min.
4. Remove the permeablization buffer and wash three times for 10 minutes each in PBS. Be sure to not let the specimen dry out.
5. To block nonspecific antibody binding, incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
6. Add primary antibody at appropriate dilution and incubate overnight at 4C.
7. Remove primary antibody and replace with PBS. Wash three times for 10 minutes each.
8. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
9. Remove secondary antibody and replace with PBS. Wash three times for 10 minutes each.
10. Counter stain DNA with DAPi if required.

Immunohistochemistry-Paraffin Protocol for TRF-2 Antibody (NB100-56506)
Immunohistochemistry-Paraffin Embedded Sections

Antigen Unmasking:
Bring slides to a boil in 10 mM sodium citrate buffer (pH 6.0) then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench-top for 30 minutes (keep slides in the sodium citrate buffer at all times).

Staining:
1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in PBS for 5 minutes.
3. Block each section with 100-400 ul blocking solution (1% BSA in PBS) for 1 hour at room temperature.
4. Remove blocking solution and add 100-400 ul diluted primary antibody. Incubate overnight at 4 C.
5. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
6. Add 100-400 ul HRP polymer conjugated secondary antibody. Incubate 30 minutes at room temperature.
7. Wash sections three times in wash buffer for 5 minutes each.
8. Add 100-400 ul DAB substrate to each section and monitor staining closely.
9. As soon as the sections develop, immerse slides in deionized water.
10. Counterstain sections in hematoxylin.
11. Wash sections in deionized water two times for 5 minutes each.
12. Dehydrate sections.
13. Mount coverslips.

Flow (Intracellular) Protocol for TRF-2 Antibody (NB100-56506)

Protocol for Flow Cytometry Intracellular Staining

Sample Preparation.

1. Grow cells to 60-85% confluency. Flow cytometry requires between 2 x 105 and 1 x 106 cells for optimal performance.
2. If cells are adherent, harvest gently by washing once with staining buffer and then scraping. Avoid using trypsin as this can disrupt certain epitopes of interest. If enzymatic harvest is required, use Accutase, Collagenase, or TrypLE Express for a less damaging option.
3. Reserve 100 uL for counting, then transfer cell volume into a 50 mL conical tube and centrifuge for 8 minutes at 400 RCF.

a. Count cells using a hemocytometer and a 1:1 trypan blue exclusion stain to determine cell viability before starting the flow protocol. If cells appear blue, do not proceed.

4. Re-suspend cells to a concentration of 1 x 106 cells/mL in staining buffer (NBP2-26247).
5. Aliquot out 100 uL samples in accordance with your experimental samples.

Tip: When cell surface and intracellular staining are required in the same sample, it is advisable that the cell surface staining be performed first since the fixation and permeabilization steps might reduce the availability of surface antigens.

Intracellular Staining.

Tip: When performing intracellular staining, it is important to use appropriate fixation and permeabilization reagents based upon the target and its subcellular location. Generally, our Intracellular Flow Assay Kit (NBP2-29450) is a good place to start as it contains an optimized combination of reagents for intracellular staining as well as an inhibitor of intracellular protein transport (necessary if staining secreted proteins). Certain targets may require more gentle or transient permeabilization protocols such as the commonly employed methanol or saponin-based methods.

Protocol for Cytoplasmic Targets:

1. Fix the cells by adding 100 uL fixation solution (such as 4% PFA) to each sample for 10-15 minutes.
2. Permeabilize cells by adding 100 uL of a permeabilization buffer to every 1 x 106 cells present in the sample. Mix well and incubate at room temperature for 15 minutes.

a. For cytoplasmic targets, use a gentle permeabilization solution such as 1X PBS + 0.5% Saponin or 1X PBS + 0.5% Tween-20.

b. To maintain the permeabilized state throughout your experiment, use staining buffer + 0.1% of the permeabilization reagent (i.e. 0.1% Tween-20 or 0.1% Saponin).

3. Following the 15 minute incubation, add 2 mL of the staining buffer + 0.1% permeabilizer to each sample.
4. Centrifuge for 1 minute at 400 RCF.
5. Discard supernatant and re-suspend in 100 uL of staining buffer + 0.1% permeabilizer.
6. Add appropriate amount of each antibody (eg. 1 test or 1 ug per sample, as experimentally determined).
7. Mix well and incubate at room temperature for 30 minutes- 1 hour. Gently mix samples every 10-15 minutes.
8. Following the primary/conjugate incubation, add 1-2 mL/sample of staining buffer +0.1% permeabilizer and centrifuge for 1 minute at 400 RCF.
9. Wash twice by re-suspending cells in staining buffer (2 mL for tubes or 200 uL for wells) and centrifuging at 400 RCF for 5 minutes. Discard supernatant.
10. Add appropriate amount of secondary antibody (as experimentally determined) to each sample.
11. Incubate at room temperature in dark for 20 minutes.
12. Add 1-2 mL of staining buffer and centrifuge at 400 RCF for 1 minute and discard supernatant.
13. Wash twice by re-suspending cells in staining buffer (2 mL for tubes or 200 uL for wells) and centrifuging at 400 RCF for 5 minutes. Discard supernatant.
14. Resuspend in an appropriate volume of staining buffer (usually 500 uL per sample) and proceed with analysis on your flow cytometer.

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Products Related to NB100-56506

HAF007	Goat anti-Mouse IgG Secondary Antibody [HRP]
NB720-B	Rabbit anti-Mouse IgG (H+L) Secondary Antibody [Biotin]
NBP1-43319-0.5mg	Mouse IgG1 Kappa Isotype Control (P3.6.2.8.1)
NB100-56506PE	TRF-2 Antibody (4A794.15) [PE]

Limitations

This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

For more information on our 100% guarantee, please visit www.novusbio.com/guarantee

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