Product Datasheet

Actin Antibody (mAbGEa)
NB100-74340

Unit Size: 0.1 ml

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

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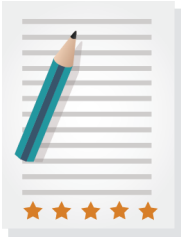
Updated 12/20/2023 v.20.1



NB100-74340

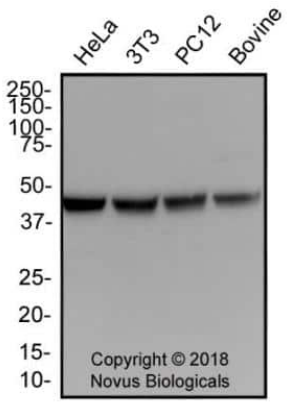
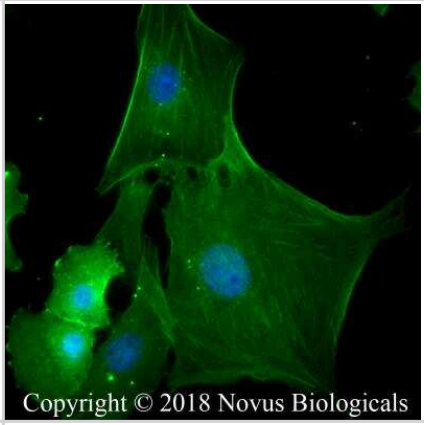
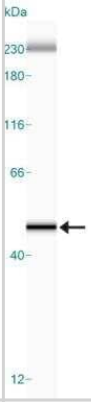
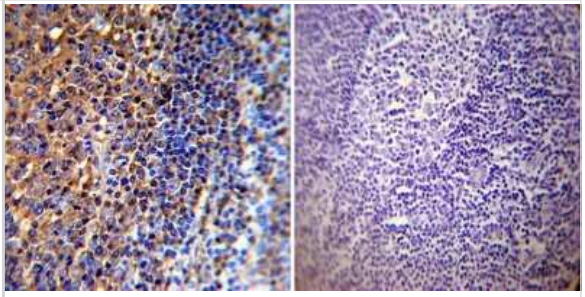
Actin Antibody (mAbGEa)

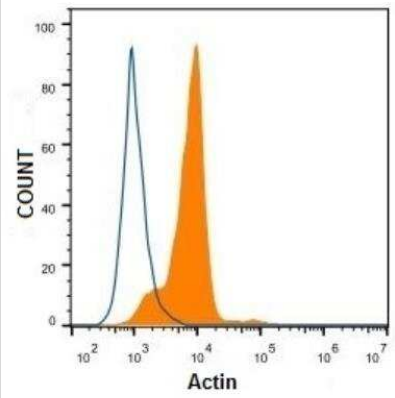
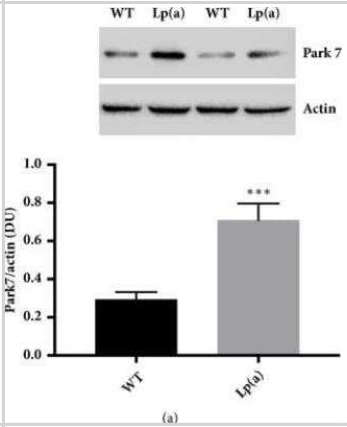
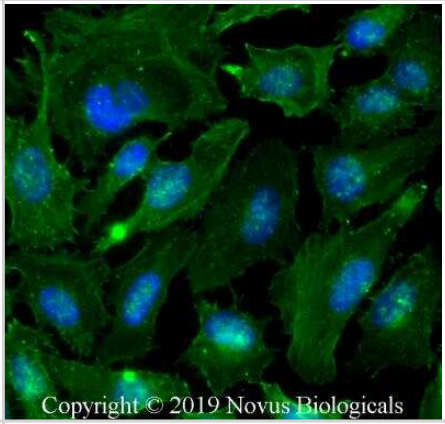
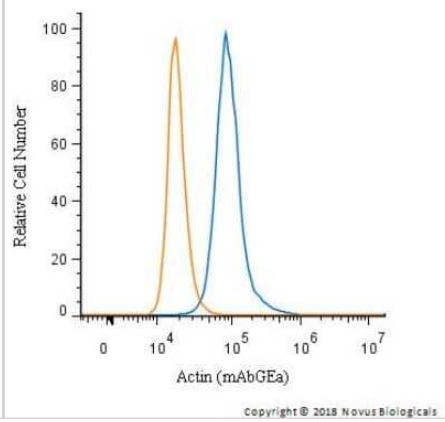
Product Information	
Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	mAbGEa
Preservative	0.05% Sodium Azide
Isotype	IgM Kappa
Purity	IgM purified
Buffer	PBS

Product Description	
Host	Mouse
Gene ID	58
Gene Symbol	ACTA1
Species	Human, Mouse, Rat, Bovine, Chinese Hamster, Drosophila, Fungi, Plant, Protozoa, Rabbit, Sheep, Xenopus, Yeast, Zebrafish
Reactivity Notes	Chinese hamster data from customer review. Sordaria macrospora reactivity reported in scientific literature (PMID: 24720701).
Specificity/Sensitivity	Reacts with Actin 1, 2, 3, 4, 7, 8, 11 and 12.
Immunogen	Purified recombinant Arabidopsis Actin protein [UniProt# P0CJ46]

Product Application Details	
Applications	Western Blot, Simple Western, ELISA, Flow Cytometry, Flow (Intracellular), Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin
Recommended Dilutions	Western Blot 1:100-1:1000, Simple Western 1:25, Flow Cytometry 1 ug/ml, ELISA 1:100 - 1:2000, Immunohistochemistry 1:200, Immunocytochemistry/ Immunofluorescence 1:20-1:100, Immunohistochemistry-Paraffin 1:200, Flow (Intracellular) 1 ug/ml
Application Notes	In Western blot, a band is seen at ~45 kDa representing Actin. 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. In IHC-P and ICC/IF, cytoplasmic staining is observed. Prior to immunostaining paraffin tissues, antigen retrieval with sodium citrate buffer (pH 6.0) is recommended. In Simple Western only 10 - 15 uL of the recommended dilution is used per data point. Separated by Size-Wes, Sally Sue/Peggy Sue.

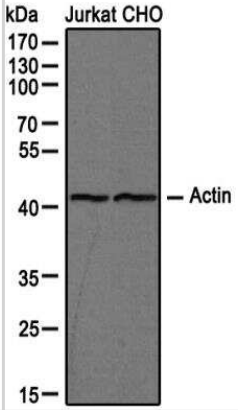
Images

Western Blot: Actin Antibody (mAbGEa) [NB100-74340] - Total protein from HeLa, 3T3, PC12 and Bovine normal tissue 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 1.0 ug/mL anti-Actin in 5% blocking buffer and detected with an anti-mouse IgM secondary antibody using chemiluminescence.	
Immunocytochemistry/Immunofluorescence: Actin Antibody (mAbGEa) [NB100-74340] - NIH-3T3 cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X PBS + 0.05% Triton X-100. The cells were incubated with anti-Actin (mAbGEa) at 5 ug/mL overnight at 4C and detected with an anti-Mouse IgM DyLight 488 (Green) at a 1:500 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.	
Simple Western: Actin Antibody (mAbGEa) [NB100-74340] - Image shows a specific band for Actin in 0.1 mg/mL of HeLa lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.	
Immunohistochemistry-Paraffin: Actin Antibody (mAbGEa) [NB100-74340] - Both normal and cancer biopsies of deparaffinized Human tonsil tissues.	

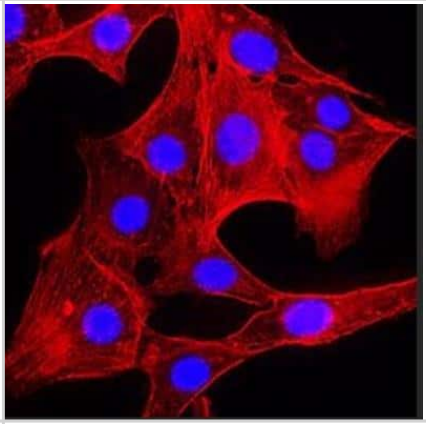
Flow Cytometry: Actin Antibody (mAbGEa) [NB100-74340] - Analysis of HeLa cells using mouse Actin antibody (Orange) and Isotype control Antibody (Blue).	
Western Blot: Actin Antibody (mAbGEa) [NB100-74340] - Increased relative abundance of Park7 in Lp(a) mice. Western blots were used to validate the comparative proteomic analysis between the Lp(a) and wildtype mice for the Park7 protein. Pooled liver protein extracts (n = 4 livers per pool) were separated by SDS PAGE in multiple replicates (n = 7) and transferred onto nitrocellulose membrane. Membranes were probed with an anti-Park7 antibody using an anti-actin antibody as a loading control. Liver protein extracts were the same as those used for Figure 4(a) as well as fresh liver protein extracts from new mice of the same age, sex, and genotype. Image collected and cropped by CiteAb from the following publication (null), licensed under a CC-BY license.	
Immunocytochemistry/Immunofluorescence: Actin Antibody (mAbGEa) [NB100-74340] - HeLa cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X PBS + 0.05% Triton-X100. The cells were incubated with anti-Actin Antibody (mAbGEa) at 2 ug/ml overnight at 4C and detected with an anti-mouse Dylight 488 (Green) at a 1:500 dilution. Actin was detected with Phalloidin 568 (Red) at a 1:200 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.	
Flow (Intracellular): Actin Antibody (mAbGEa) [NB100-74340] - An intracellular stain was performed on A549 cells with NB100-74340 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeablized with 0.1% saponin. Cells were incubated in an antibody dilution of 1 ug/mL for 30 minutes at room temperature, followed by mouse IgM Alexa Fluor 488-conjugated secondary antibody.	

Western Blot: Actin Antibody (mAbGEa) [NB100-74340] - Analysis of Actin expression in 2) HeLa, 3) NTERA-2, 4) A431, 5) HepG2, 6) MCF7, 7) NIH-3T3, 8) PC-12 and 9) COS-7 whole cell lysates.

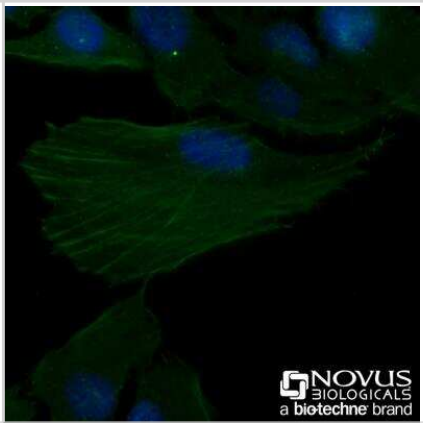
Western Blot: Actin Antibody (mAbGEa) [NB100-74340] - Analysis of Jurkat and CHO cell lysates using actin antibody [NB100-74340] at 1:100.

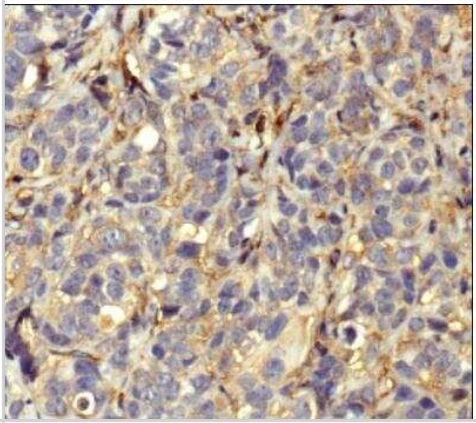
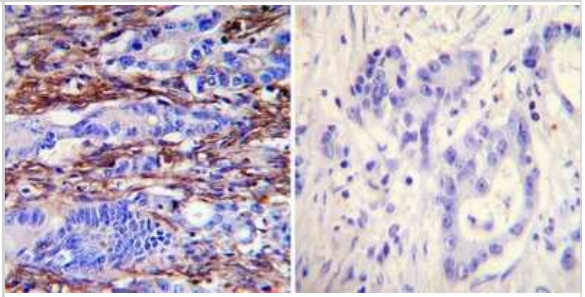
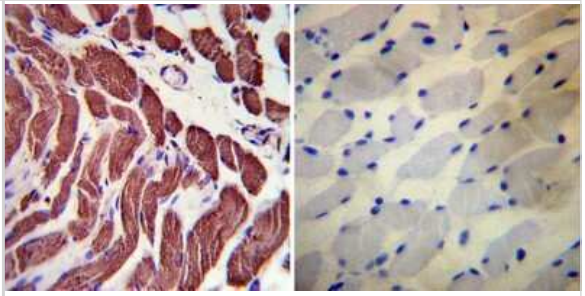
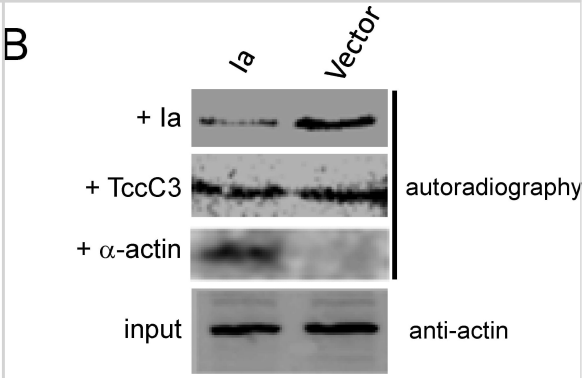


Immunocytochemistry/Immunofluorescence: Actin Antibody (mAbGEa) [NB100-74340] - Actin was detected in NIH-3T3 cells fixed with methanol using mouse anti-mouse beta-Actin monoclonal antibody (NB100-74340) at 1:1800. Cells were stained using Northern Lights 557 conjugated anti-mouse secondary antibody (NL007) and counterstained with DAPI.

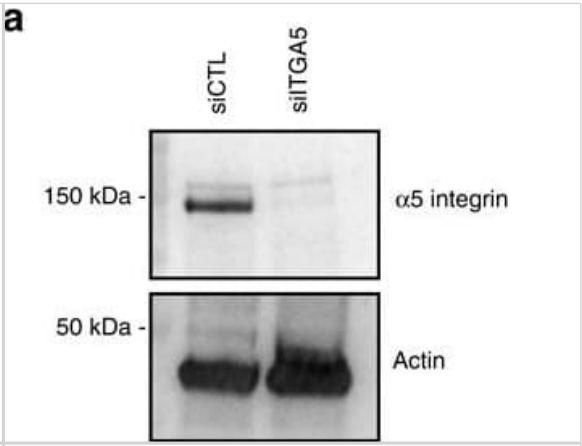


Immunocytochemistry/Immunofluorescence: Actin Antibody (mAbGEa) [NB100-74340] - HeLa cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X PBS + 0.05% Triton X-100. The cells were incubated with anti-Actin (mAbGEa) at 2 ug/mL overnight at 4C and detected with an anti-mouse IgM DyLight 488 (Green) at 1:500. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.

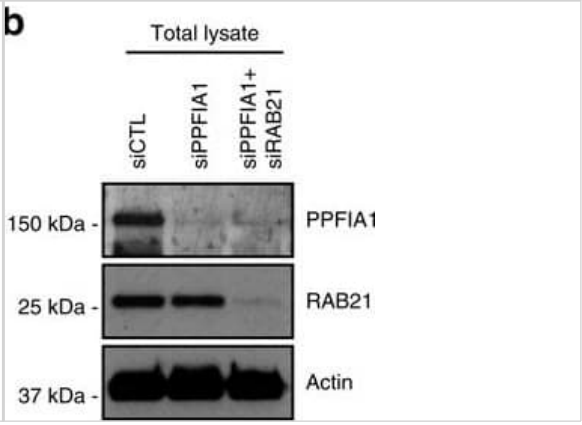


Immunohistochemistry-Paraffin: Actin Antibody (mAbGEa) [NB100-74340] - Analysis of Actin on human breast cancer tissue using DAB with hematoxylin counterstain.	
Immunohistochemistry-Paraffin: Actin Antibody (mAbGEa) [NB100-74340] - Both normal and cancer biopsies of deparaffinized Human colon carcinoma tissues.	
Immunohistochemistry-Paraffin: Actin Antibody (mAbGEa) [NB100-74340] - Both normal and cancer biopsies of deparaffinized Human skeletal muscle tissues.	
Analysis of Ia production by engineered S. cerevisiae. (B) Production of Ia by the wild type S. cerevisiae strain. Wild type yeast strains harboring the Ia-containing plasmid (Ia) or the control vector (vector) were cultivated in glucose-containing liquid medium until OD595 = 0.5. Afterwards, glucose was replaced by galactose and cultivation continued for 9 h at 30C. Cells were lysed and the resulting extract preparations were ADP-ribosylated in the presence of Ia (+ Ia), TccC3 toxin of P. luminescens [42] (+ TccC3), purified muscle actin (+alpha-actin) or tested in Western blotting with the anti-actin serum to show equal actin concentrations in the samples. Image collected and cropped by CiteAb from the following publication (https://dx.plos.org/10.1371/journal.pone.0145708), licensed under a CC-BY licence.	

UHRF1 deficiency resulted in impaired meiotic recombination and defective pachynema.a Double immunofluorescence of SYCP3 (green) and DMC1 (red) in testicular spread preparations. b, c The number of DMC1 foci in zygotene stage (b) and pachytene stage (c). d Immunostaining for SYCP3 (red) and γH2AX (green). e The percentage of abnormal γH2AX foci in the pachytene stage. f Immunostaining for SYCP3 (red) and MLH1 (green). g The number of MLH1 foci in pachynema. h Immunostaining for SYCP3 (red) and H1t (green). i The percentage of spermatocytes with H1T staining. ***p ≤ 0.001; *p ≤ 0.05. Scale bar, 5 μm in a, d, f, h. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/32081844), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



IMR90 cells depleted for Ajuba undergo a DNA damage response. (A,C,D) Western blots for Ajuba, Cyclin A, Rb, Chk1 phosphorylation, and PARP cleavage on lysates prepared from IMR90 cells 72 h after transfection with siRNA #3. The loading controls with GAPDH for each blot are shown. (B) Staining for p53BP1 in Ajuba-depleted cells (siRNA#3), with siGFP as a control. Image collected and cropped by CiteAb from the following open publication (https://pubmed.ncbi.nlm.nih.gov/23755068), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Wang C, Zhang Y, Zhou B et al. Modifications of Two ESCRT-I Subunits with Distinct Ubiquitin Chains Regulate Plant Immunity bioRxiv 2023-08-16 (WB, Plant)

Details:
Arabidopsis thaliana

Hamann A, Osiewacz HD, Teichert I. Sordaria macrospora Sterile Mutant pro34 Is Impaired in Respiratory Complex I Assembly Journal of Fungi 2022-09-27 [PMID: 36294581] (WB)

Biehler C, Rothenberg Ke, Jette A Et Al. Pak1 and PP2A antagonize aPKC function to support cortical tension induced by the Crumbs-Yurt complex eLife 2021-07-02 [PMID: 34212861] (WB)

Biehler C, Rothenberg K, Jetté A, et al. Functional plasticity of polarity proteins controls epithelial tissue architecture bioRxiv 2021-01-06 (WB, Drosophila)

Frey S, Lahmann Y et al. Deletion of Smgpi1 encoding a GPI-anchored protein suppresses sterility of the STRIPAK mutant delta Smmob3 in the filamentous ascomycete Sordaria macrospora. Mol Microbiol 2015-01-08 [PMID: 25989468] (WB, Fungus)

Details:
Sordaria macrospora

Belyy A, Tabakova I et al. Roles of Asp179 and Glu270 in ADP-Ribosylation of Actin by Clostridium perfringens Iota Toxin. PLoS One 2015-12-30 [PMID: 26713879] (WB, Yeast)

Details:
Saccharomyces cerevisiae (Yeast)

Rodger E J, Porteous C M et al. Proteomic Analysis of Liver from Human Lipoprotein(a) Transgenic Mice Shows an Oxidative Stress and Lipid Export Response. Biomed Res Int 2019-01-01 [PMID: 30596094] (WB, Mouse, Human)

Redhu AK, Bhat JP Mitochondrial glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase abrogate p53 induced apoptosis in a yeast model: Possible implications for apoptosis resistance in cancer cells Biochim Biophys Acta Gen Subj 2019-12-17 [PMID: 31862471] (WB, Yeast)

Redhu AK, Bhat JP Novel Proteomic changes in Yeast Mitochondria provide insights into mitochondrial functioning upon over-expression of human p53 bioRxiv 2019-08-24 (WB, Yeast)

Taylor SM, Giuffre E, Moseley P, Hitchcock PF The MicroRNA, miR-18a, Regulates NeuroD and Photoreceptor Differentiation in the Retina of Zebrafish Dev Neurobiol 2019-02-01 [PMID: 30615274] (WB, Zebrafish)

Audesse, AJ;Dhakal, S;Hassell, LA;Gardell, Z;Nemtsova, Y;Webb, AE; FOXO3 directly regulates an autophagy network to functionally regulate proteostasis in adult neural stem cells PLoS Genet. 2019-04-01 [PMID: 30973875] (WB, Mouse)

Zhao, SB;Suda, Y;Nakanishi, H;Wang, N;Yoko-O, T;Gao, XD;Fujita, M; Yeast Dop1 is required for glycosyltransferase retrieval from the trans-Golgi network Biochim Biophys Acta Gen Subj 2019-06-01 [PMID: 30981741] (WB, Yeast)

More publications at http://www.novusbio.com/NB100-74340

Procedures

Western Blot Protocol Specific for Actin Antibody (mAbGEa) [NB100-74340]

Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 40 ug of total protein per lane.
2. Transfer proteins to membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain according to standard Ponceau S procedure (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot.
5. Block the membrane using standard blocking buffer for at least 1 hour.
6. Wash the membrane in wash buffer three times for 10 minutes each.
7. Dilute primary antibody in blocking buffer and incubate 1 hour at room temperature.
8. Wash the membrane in wash buffer three times for 10 minutes each.
9. Apply the diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturers instructions) and incubate 1 hour at room temperature.
10. Wash the blot in wash buffer three times for 10 minutes each (this step can be repeated as required to reduce background).
11. Apply the detection reagent of choice in accordance with the manufacturers instructions.

Note: Tween-20 can be added to the blocking or antibody dilution buffer at a final concentration of 0.05-0.2%.

*The above information is only intended as a guide. The researcher should determine what protocol best meets their needs. Please follow safe laboratory procedures.

Immunohistochemistry protocol for Actin Antibody (NB100-74340)

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.

Staining:
1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in wash buffer for 5 minutes.
3. Block each section with 100-400 ul blocking solution 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 biotinylated diluted secondary antibody. Incubate 30 minutes at room temperature.
7. Remove secondary antibody solution and wash sections three times with wash buffer for 5 minutes each.
8. Add 100-400 ul Streptavidin-HRP reagent to each section and incubate for 30 minutes at room temperature.
9. Wash sections three times in wash buffer for 5 minutes each.
10. Add 100-400 ul DAB substrate to each section and monitor staining closely.
11. As soon as the sections develop, immerse slides in deionized water.
12. Counterstain sections in hematoxylin.
13. Wash sections in deionized water two times for 5 minutes each.
14. Dehydrate sections.
15. Mount coverslips.

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Immunocytochemistry/Immunofluorescence protocol for Actin Antibody (NB100-74340)

Immunocytochemistry Protocol

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

1. Remove culture medium and add 10% formalin to the dish. Fix at room temperature for 30 minutes.
2. Remove the formalin and add ice cold methanol. Incubate for 5-10 minutes.
3. Remove methanol and add washing solution (i.e. PBS). Be sure to not let the specimen dry out. Wash three times for 10 minutes.
4. To block nonspecific antibody binding incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
5. Add primary antibody at appropriate dilution and incubate at room temperature from 2 hours to overnight at room temperature.
6. Remove primary antibody and replace with washing solution. Wash three times for 10 minutes.
7. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
8. Remove antibody and replace with wash solution, then wash for 10 minutes. Add Hoechst 33258 to wash solution at 1:25,0000 and incubate for 10 minutes. Wash a third time for 10 minutes.
9. Cells can be viewed directly after washing. The plates can also be stored in PBS containing Azide covered in Parafilm (TM). Cells can also be cover-slipped using Fluoromount, with appropriate sealing.

*The above information is only intended as a guide. The researcher should determine what protocol best meets their needs. Please follow safe laboratory procedures.

Flow (Intracellular) Protocol for Actin Antibody (NB100-74340)

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 permeablization 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 permeabization 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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General: novus@novusbio.com

Products Related to NB100-74340

NBP1-42569	HepG2 Whole Cell Lysate
HAF007	Goat anti-Mouse IgG Secondary Antibody [HRP]
NB720-B	Rabbit anti-Mouse IgG (H+L) Secondary Antibody [Biotin]
NBP1-96975-0.5mg	Mouse IgM Kappa Light Chain Isotype Control (MMK)

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.

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