Product Datasheet

Apolipoprotein E/ApoE Antibody (WUE-4) - BSA Free
NB110-60531SS

Unit Size: 0.025 ml

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

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Reviews: 2 Publications: 38

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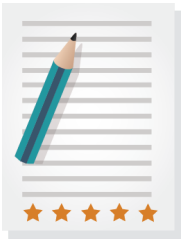
Updated 2/22/2023 v.20.1



NB110-60531SS

Apolipoprotein E/ApoE Antibody (WUE-4) - BSA Free

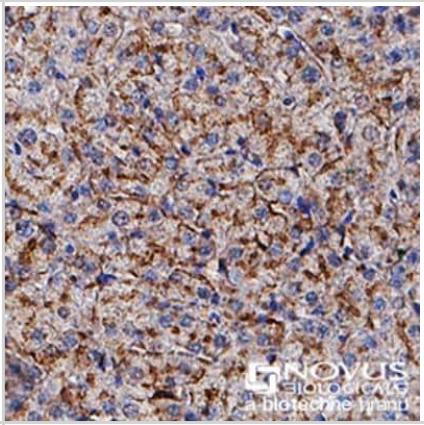
Product Information	
Unit Size	0.025 ml
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	WUE-4
Preservative	0.05% Sodium Azide
Isotype	IgG1 Kappa
Purity	Protein G purified
Buffer	PBS
Target Molecular Weight	36 kDa

Product Description	
Host	Mouse
Gene ID	348
Gene Symbol	APOE
Species	Human, Mouse, Rat (Negative)
Reactivity Notes	Reacts with human better than mouse. It does not appear to react with rat brain tissue.
Specificity/Sensitivity	This antibody detects ApoE2, ApoE3 and ApoE4 (PMID 2466929).
Immunogen	Purified human ApoE [UniProt# P02649]

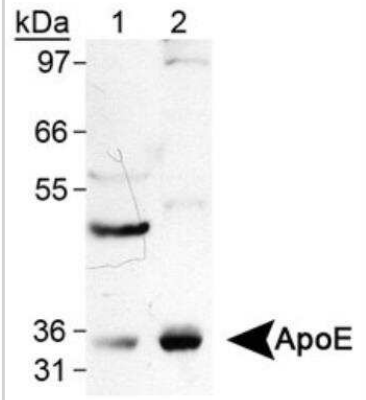
Product Application Details	
Applications	Western Blot, ELISA, Flow Cytometry, Flow (Intracellular), Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation, CyTOF-ready
Recommended Dilutions	Western Blot 2 ug/ml, Flow Cytometry 1 ug per million cells, ELISA 1:100-1:2000, Immunohistochemistry 1:50-1:200, Immunocytochemistry/ Immunofluorescence 1:200, Immunoprecipitation 1:10-1:500, Immunohistochemistry-Paraffin, Flow (Intracellular), CyTOF-ready
Application Notes	This ApoE antibody is useful for Western blot, ELISA, Immunohistochemistry and Immunoprecipitation. In Western blot a band is observed at ~36 kDa, representing the ApoE protein. 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. Use in Immunocytochemistry/immunofluorescence reported in scientific literature (PMID: 26564908). This antibody is CyTOF ready.

Images

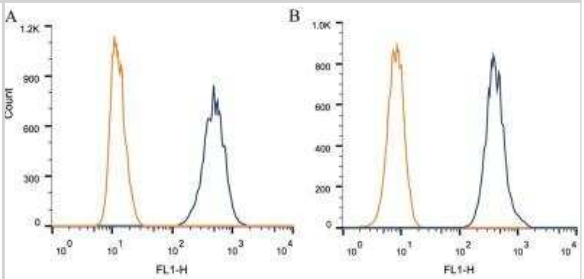
Immunohistochemistry-Paraffin: Apolipoprotein E/ApoE Antibody (WUE-4) [NB110-60531] - ApoE was detected in immersion fixed paraffin-embedded sections of human liver using anti-human mouse monoclonal antibody (Catalog # NB110-60531) at 1:200 dilution overnight at 4C. Tissue was stained using the VisuCyte anti-mouse HRP polymer detection reagent (Catalog # VC001) with DAB chromogen (brown) and counterstained with hematoxylin (blue). Images may not be copied, printed or otherwise disseminated without express written permission of Novus Biologicals a bio-techne brand.



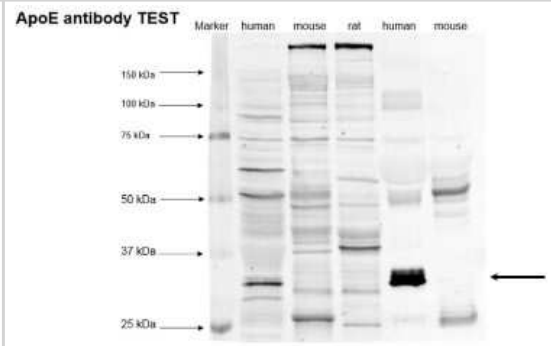
Western Blot: Apolipoprotein E/ApoE Antibody (WUE-4) [NB110-60531] - ApoE Antibody (WUE-4) [NB110-60531] - Detection of ApoE in human tissue lysate using NB110-60531. Lane 1: liver Lane 2: brain



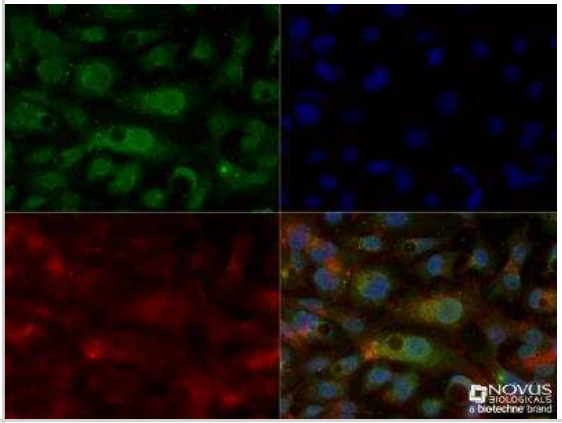
Flow Cytometry: Apolipoprotein E/ApoE Antibody (WUE-4) [NB110-60531] - ApoE Antibody (WUE-4) [NB110-60531] - Intracellular flow cytometric staining of 1 x 10^6 CHO (A) and HEK-293 (B) cells using ApoE antibody (dark blue). Isotype control shown in orange. An antibody concentration of 1 ug/1x10^6 cells was used.



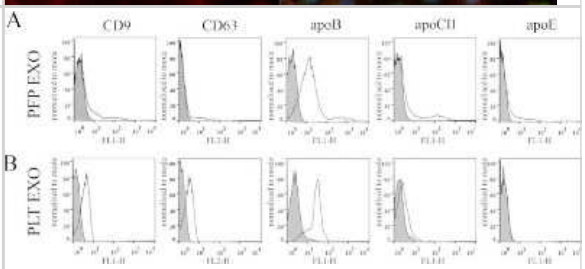
Western Blot: Apolipoprotein E/ApoE Antibody (WUE-4) [NB110-60531] - 50 ug protein per lane for liver lysate and 0.5 ul of plasma at 9% SDS. Samples are loaded onto the gel as the following order: human liver lysate, mouse liver lysate, rat liver lysate, human plasma, and mouse plasma. Image from confirmed customer review.



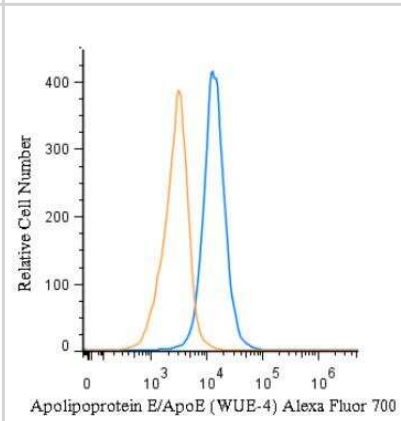
Immunocytochemistry/Immunofluorescence: Apolipoprotein E/ApoE Antibody (WUE-4) [NB110-60531] - HepG2 cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X TBS + 0.5% Triton X-100. The cells were incubated with anti-ApoE (WUE-4) [NB110-60531] at a 1:200 dilution 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.



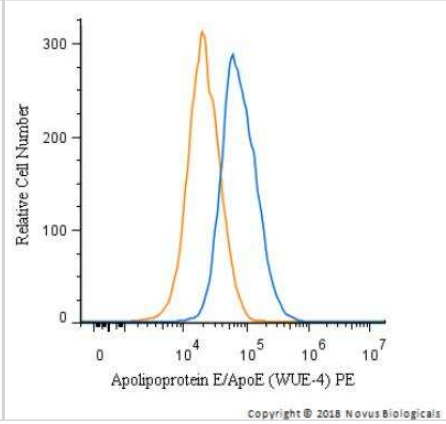
Flow Cytometry: Apolipoprotein E/ApoE Antibody (WUE-4) [NB110-60531] - Analysis of apoB-positivity in blood plasma and PLT concentrate-derived EXOs. FCM detection of the indicated markers in EXOs conjugated onto latex beads. The EXOs were isolated from fasting PFP or PLT concentrate by differential UC and gravity size filtration (gray histograms: blocked beads incubated with antibody, empty histograms: EXO sample). Image collected and cropped by CiteAb from the following publication (https://www.nature.com/articles/srep24316), licensed under a CC-BY license.

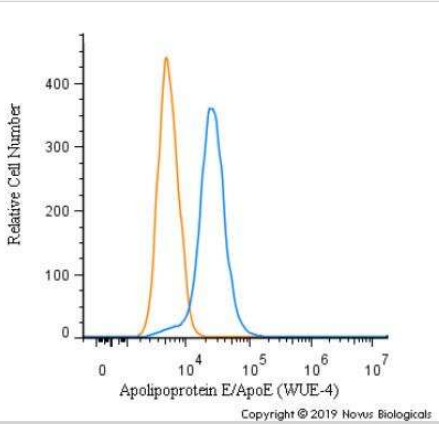
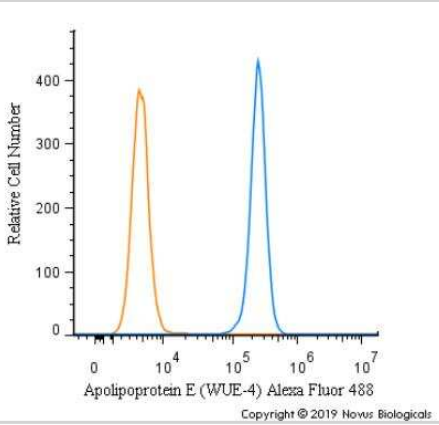
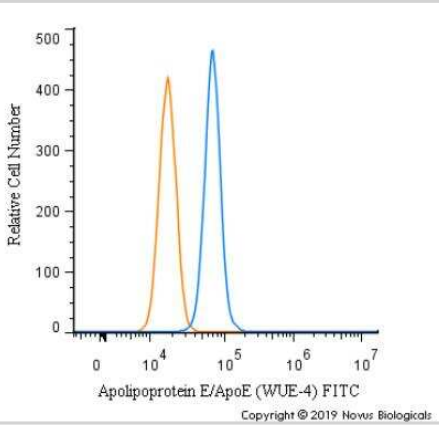
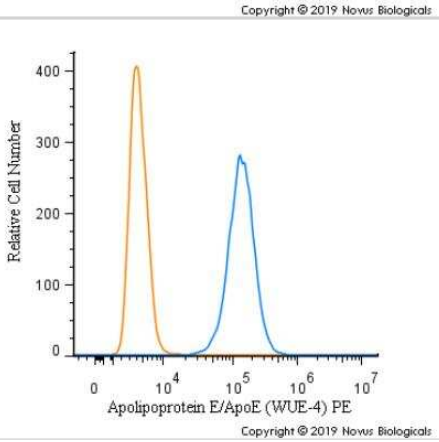


Flow (Intracellular): Apolipoprotein E/ApoE Antibody (WUE-4) [NB110-60531] - An intracellular stain was performed on HepG2 cells with NB110-60531AF700 (blue) and a matched isotype control (orange). Cells were fixed with 4% PFA and then permeablized with 0.1% saponin. Cells were incubated in an antibody dilution of 5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 700.



Flow Cytometry: Apolipoprotein E/ApoE Antibody (WUE-4) [NB110-60531] - An intracellular stain was performed on HepG2 cells with NB110-60531PE (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 5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Phycoerythrin.



Flow Cytometry: Apolipoprotein E/ApoE Antibody (WUE-4) [NB110-60531] - An intracellular stain was performed on HepG2 cells with Apolipoprotein E/ApoE Antibody NB110-60531 [WUE-4] (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 488.	
Flow Cytometry: Apolipoprotein E/ApoE Antibody (WUE-4) [NB110-60531] - An intracellular stain was performed on SK-MEL-28 cells with alpha-Synuclein [2A7] Antibody NB110-60531AF488 (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 5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Alexa Fluor 488.	
Flow Cytometry: Apolipoprotein E/ApoE Antibody (WUE-4) [NB110-60531] - An intracellular stain was performed on SK-MEL-28 cells with Apolipoprotein E/ApoE Antibody [WUE-4] NB110-60531F (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 10 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to FITC.	
Flow Cytometry: Apolipoprotein E/ApoE Antibody (WUE-4) [NB110-60531] - An intracellular stain was performed on SK-MEL-28 cells with Apolipoprotein E/ApoE [WUE-4] Antibody NB110-60531PE (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 2.5 ug/mL for 30 minutes at room temperature. Both antibodies were conjugated to Phycoerythrin.	

Publications

Camila Fernández Zapata, Ginevra Giacomello, Eike J. Spruth, Jinte Middeldorp, Gerardina Gallaccio, Adeline Dehlinger, Claudia Dames, Julia K. H. Leman, Roland E. van Dijk, Andreas Meisel, Stephan Schlickeiser, Desiree Kunkel, Elly M. Hol, Friedemann Paul, Maria Kristina Parr, Josef Priller, Chotima Böttcher Differential compartmentalization of myeloid cell phenotypes and responses towards the CNS in Alzheimer's disease Nature Communications 2022-11-23 [PMID: 36418303]

A Jeyaram, TN Lamichhane, S Wang, L Zou, E Dahal, SM Kronstadt, D Levy, B Parajuli, DR Knudsen, W Chao, SM Jay Enhanced Loading of Functional miRNA Cargo via pH Gradient Modification of Extracellular Vesicles Mol. Ther., 2019-12-24;0(0):. 2019-12-24 [PMID: 31911034]

Watanabe H, Murakami R, Tsumagari K et al. Astrocytic APOE4 genotype-mediated negative impacts on synaptic architecture in human pluripotent stem cell model Stem cell reports 2023-09-12 [PMID: 37657448] (ELISA)

Details:
Supplemental information: plates were coated overnight with NB110-60531, in 0.1 M carbonate buffer at 4?°C

Konings SC, Torres-Garcia L, Martinsson I, Gouras GK. Astrocytic and Neuronal Apolipoprotein E Isoforms Differentially Affect Neuronal Excitability Frontiers in Neuroscience 2021-09-21 [PMID: 34621153] (ICC/IF, WB)

Jung JS, Volk C, Marga C et al. Adipose-Derived Stem/Stromal Cells Recapitulate Aging Biomarkers and Show Reduced Stem Cell Plasticity Affecting Their Adipogenic Differentiation Capacity Cellular Reprogramming 2019-08-01 [PMID: 31298565]

Kerr T The Effects of Exercise on the Lipid Profile of Extracellular Vesicles Thesis 2023-01-01 (WB, Human)

Kessler K, Giannisis A, Bial G et al. Behavioral and cognitive performance of humanized APOE?3/?3 liver mice in relation to plasma apolipoprotein E levels Scientific reports 2023-01-31 [PMID: 36720957] (ELISA, Mouse)

Watanabe S, Sudo Y, Makino T, Kimura S Skeletal muscle releases extracellular vesicles with distinct protein and miRNA signatures that function in the muscle microenvironment PNAS 2022-01-01 [PMID: 36714847] (WB, Mouse)

Zhang H, Shao L, Lin Z et al. APOE interacts with ACE2 inhibiting SARS-CoV-2 cellular entry and inflammation in COVID-19 patients Signal transduction and targeted therapy 2022-08-01 [PMID: 35915083] (ICC/IF)

Milich LM, Choi JS, Ryan CB et al. Single cell analysis of the cellular heterogeneity and interactions in the injured mouse spinal cord J Exp Med 2021-06-16 [PMID: 34132743]

Jin Y, Li F, Sonoustoun B et al. APOE4 exacerbates alpha-synuclein seeding activity and contributes to neurotoxicity in Alzheimer's disease with Lewy body pathology Acta neuropathologica 2022-04-26 [PMID: 35471463] (ELISA, Human)

Konings S The synaptic and neurobiological role of apolipoprotein E4 in models of Alzheimer's disease Thesis 2022-01-01 (WB, Human)

More publications at http://www.novusbio.com/NB110-60531

Procedures

Western Blot Protocol for ApoE Antibody (NB110-60531)

Western Blot Protocol

1. Perform SDS-PAGE (4-12% MOPS) on samples to be analyzed, loading 40ug of total protein per lane.
2. Transfer proteins to Nitrocellulose according to the instructions provided by the manufacturer of the transfer apparatus.
3. Rinse membrane with dH2O and then stain the blot using Ponceau S for 1-2 minutes to access the transfer of proteins onto the nitrocellulose membrane. Rinse the blot in water to remove excess stain and mark the lane locations and locations of molecular weight markers using a pencil.
4. Rinse the blot in TBS for approximately 5 minutes.
5. Block the membrane using 5% non-fat dry milk + 1% BSA in TBS, 1 hour at room temperature.
6. Rinse the membrane in dH2O and then wash the membrane in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each.
7. Dilute the mouse anti-ApoE primary antibody (NB 110-60531) in blocking buffer and incubate 1 hour at room temperature.
8. Rinse the membrane in dH2O and then wash the membrane in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each.
9. Apply the diluted mouse-IgG HRP-conjugated secondary antibody in blocking buffer (as per manufacturer's instructions) and incubate 1 hour at room temperature.
10. Wash the blot in wash buffer [TBS + 0.1% Tween] 3 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 (Pierce ECL).
Note: Tween-20 can be added to the blocking or antibody dilution buffer at a final concentration of 0.05-0.2%, provided it does not interfere with antibody-antigen binding.

Immunocytochemistry/Immunofluorescence Protocol for Apolipoprotein E/ApoE Antibody (NB110-60531)
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.

Flow (Intracellular) Protocol for Apolipoprotein E/ApoE Antibody (NB110-60531)

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.

Immunohistochemistry-Paraffin Protocol for Apolipoprotein E/ApoE Antibody (NB110-60531)
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 all the time).

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.

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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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