Quantikine® ELISA

Human Apolipoprotein E/ApoE

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Human Apolipoprotein E/ApoE Immunoassay

Catalog Number DAPE00

For the quantitative determination of human Apolipoprotein E (ApoE) concentrations in cell culture supernates, serum, plasma, saliva, urine, and human milk.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Apolipoprotein E (ApoE) is a major protein component of serum LDL, VLDL, HDL, and chylomicrons and also plays a critical role in amyloid metabolism and the development of Alzheimer's disease. It is produced predominantly by hepatocytes, macrophages, and non-neuronal cells in the central nervous system. ApoE-containing particles transport triglycerides and cholesterol to peripheral tissues for cellular uptake and catabolism followed by reverse transport of HDL and chylomicron remnants to the liver (1-3). Mature human ApoE is a 34 kDa glycoprotein that consists of an N-terminal domain of four bundled α-helices, a hinge region, and an extended α-helical C-terminal domain (2, 4). Its amphipathic nature and flexible structure enable it to adopt a dramatically different conformation upon lipid association (2). ApoE is monomeric in lipid particles, although it forms oligomers when lipid-free (5, 6). ApoE3 is the most abundant of three common alleles in human; ApoE2 and ApoE4 differ by single amino acid (aa) substitutions which induce significant structural changes (2). Mature human ApoE shares 71% aa sequence identity with mouse and rat ApoE.

Most LDL receptor family proteins preferentially bind and internalize the lipid-bound form of ApoE. VLDLR also efficiently internalizes lipid-free ApoE (7, 8). Lipoprotein uptake is facilitated by the initial binding of ApoE to cell surface heparan sulfate proteoglycans (HSPG) (9). Receptor/HSPG binding and lipid interactions primarily involve the N- and C-terminal regions of ApoE, respectively (2). Recycling lipid-free ApoE is formed into HDL particles through interactions with the lipid transporter ABCA1 (10). High cellular sterol content activates the nuclear hormone receptor LXR which promotes increased ApoE synthesis and increased sterol efflux, while low sterol content induces LDL R expression, increased sterol uptake, and decreased ApoE production (11). ApoE suppresses TNF-α, TLR3, and TLR4 mediated inflammation (12, 13) and promotes the development of regulatory T cells and anti-inflammatory M2 macrophages (14, 15).

Differences in the effects of ApoE isoforms on cholesterol and amyloid Aβ peptide metabolism underlie the association between ApoE4 expression and metabolic syndrome, type II diabetes, atherosclerosis, and Alzheimer's disease (1, 16). ApoE blocks production of the amyloid Aβ peptide by inhibiting the γ-secretase cleavage of APP (17). In the cerebrospinal fluid, ApoE2 and ApoE3 bind to Aβ (16, 18, 19), while ApoE4 is much weaker (6, 20). ApoE2 and ApoE3 support the integrity of the blood brain barrier (BBB), while ApoE4 can trigger pericyte inflammation and BBB breakdown (21). The ApoE4 allele selectively impairs synaptic plasticity and NMDA R activation by Reelin (22).

ApoE can be cleaved into several fragments which enter the neuronal cytosol and mitochondria (23), and this degradation is enhanced by excitotoxic stimulation (24). Neuron-specific cleavage of ApoE is associated with increased tau phosphorylation and aggregation (24). Of the three alleles, ApoE4 is most prone to fragmentation (20). Some ApoE fragments induce mitochondrial dysfunction and neurotoxicity, while other fragments and the full length molecule do not (23). Fragments of ApoE4 preferentially accumulate in Alzheimer's disease plaques (20, 24).

The Quantikine Human Apolipoprotein E/ApoE Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human ApoE in cell culture supernates, serum, plasma, saliva, urine, and human milk. It contains *E. coli*-expressed recombinant human ApoE and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human ApoE showed linear curves that were parallel to the standard curves obtained using the Quantikine kit standards. These results indicate that this kit can be used to determine relative mass values for naturally occurring human ApoE.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A polyclonal antibody specific for human ApoE has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any ApoE present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for human ApoE is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of ApoE bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, further dilute the samples with Calibrator Diluent and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human ApoE Microplate	894638	96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody specific for human ApoE.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
Human ApoE Conjugate	894639	21 mL of a monoclonal antibody specific for human ApoE conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human ApoE Standard	894640	Recombinant human ApoE in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Assay Diluent RD1-113	895983	21 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5P Concentrate	895151	21 mL of a buffered protein base with preservatives. *Use diluted 1:5 in this assay.*	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	6 mL of 2 N sulfuric acid.	
Plate Sealers	N/A	4 adhesive strips.	

* Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- **Polypropylene** test tubes for dilution of standards and samples.
- Human ApoE Controls (optional; R&D Systems, Catalog # QC45).

PRECAUTIONS

ApoE is detectable in saliva. Take precautionary measures to prevent contamination of kit reagents while running this assay.

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Please refer to the MSDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Citrate plasma has not been validated for use in this assay.*

Saliva - Collect saliva in a tube and centrifuge for 5 minutes at 10,000 x g. Collect the aqueous layer, and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, and assay immediately or aliquot and store at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Human Milk - Centrifuge for 15 minutes at 1000 x g at 2-8 °C. Collect the aqueous fraction and repeat this process a total of 3 times. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Serum and plasma samples require a 500-fold dilution. A suggested 500-fold dilution can be achieved by adding 30 µL of sample to 570 µL of Calibrator Diluent RD5P (diluted 1:5)*. Complete the 500-fold dilution by adding 20 µL of the diluted sample to 480 µL Calibrator Diluent RD5P (diluted 1:5).

Human milk samples require a 10-fold dilution. A suggested 10-fold dilution is 50 µL of sample + 450 µL of Calibrator Diluent RD5P (diluted 1:5).

* See Sample Preperation section.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Note: *ApoE is found in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

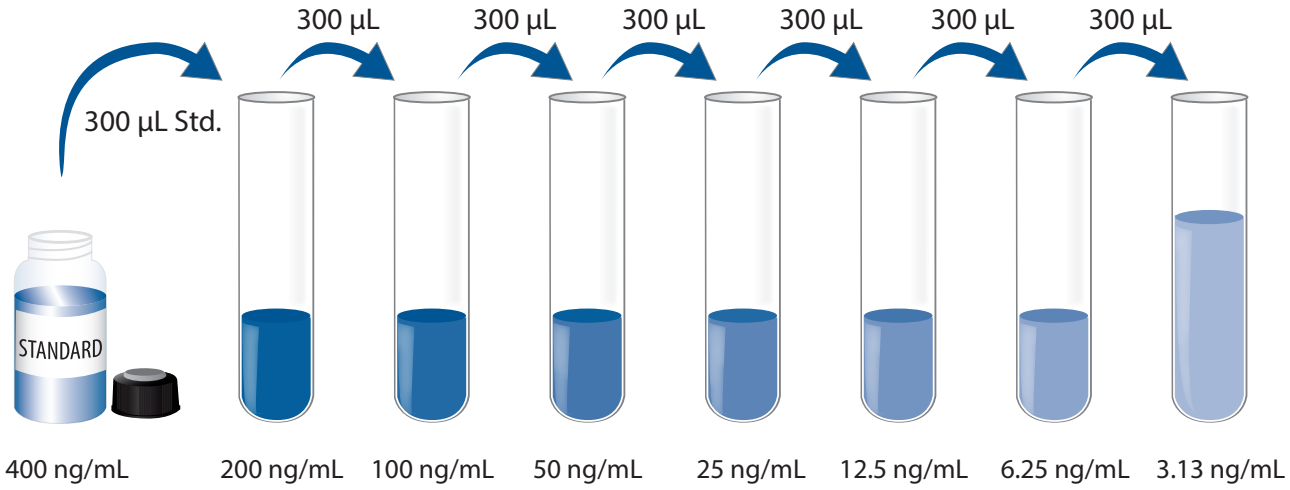
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

Calibrator Diluent RD5P (diluted 1:5) - Add 20 mL of Calibrator Diluent RD5P Concentrate to 80 mL of deionized or distilled water to prepare 100 mL of Calibrator Diluent RD5P (diluted 1:5).

Human ApoE Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human ApoE Standard with deionized or distilled water. This reconstitution produces a stock solution of 400 ng/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Use polypropylene tubes. Pipette 300 µL of Calibrator Diluent RD5P (diluted 1:5) into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 200 ng/mL standard serves as the high standard. Calibrator Diluent RD5P (diluted 1:5) serves as the zero standard (0 ng/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples, controls, and standards be assayed in duplicate.

Note: *ApoE is found in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100 µL of Assay Diluent RD1-113 to each well.
4. Add 50 µL of Standard, control, or sample* per well. Cover with the adhesive strip provided. Incubate for 3 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 200 µL of Human ApoE Conjugate to each well. Cover with a new adhesive strip. Incubate for 1 hour at room temperature on the shaker.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
9. Add 50 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

*Sample may require dilution. See the Sample Preparation section.

CALCULATION OF RESULTS

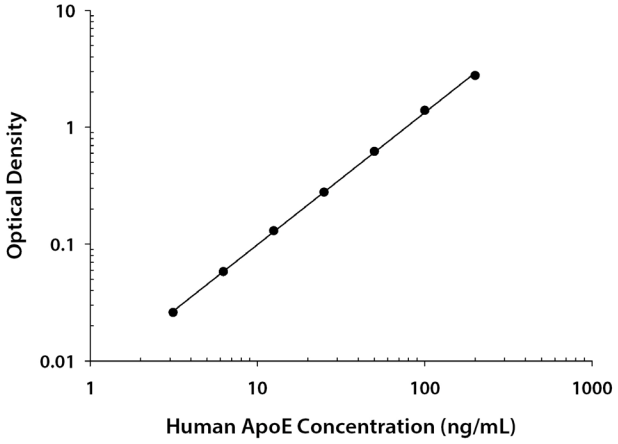
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a log/log curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on a log/log graph. The data may be linearized by plotting the log of the human ApoE concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(ng/mL)	O.D.	Average	Corrected
0	0.008 0.009	0.009	—
3.13	0.035 0.035	0.035	0.026
6.25	0.066 0.068	0.067	0.058
12.5	0.138 0.140	0.139	0.130
25	0.285 0.289	0.287	0.278
50	0.627 0.635	0.631	0.622
100	1.380 1.420	1.400	1.391
200	2.727 2.833	2.780	2.771

PRECISION

Intra-assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)
Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (ng/mL)	29.1	59.6	118	28.1	60.6	119
Standard deviation	0.913	1.38	2.91	1.71	4.17	3.89
CV (%)	3.1	2.3	2.5	6.1	6.9	3.3

RECOVERY

The recovery of human ApoE spiked to levels throughout the range of the assay was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	105	93-110%

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human ApoE were diluted with Calibrator Diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=4)	Serum* (n=4)	EDTA plasma* (n=4)	Heparin plasma* (n=4)	Human milk* (n=4)
1:2	Average % of Expected	101	104	106	100	101
	Range (%)	99-107	96-108	104-110	96-105	98-105
1:4	Average % of Expected	98	102	102	97	94
	Range (%)	92-105	98-104	96-108	95-100	87-103
1:8	Average % of Expected	98	101	101	95	92
	Range (%)	91-108	97-104	97-106	91-99	86-98
1:16	Average % of Expected	95	100	99	93	89
	Range (%)	87-105	97-101	97-102	90-95	80-99

*Samples were diluted prior to assay.

SENSITIVITY

Twenty-seven assays were evaluated and the minimum detectable dose (MDD) of human ApoE ranged from 0.067-0.709 ng/mL. The mean MDD was 0.242 ng/mL.

The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified *E. coli*-expressed recombinant human Apolipoprotein E3/ApoE3 manufactured at R&D Systems.

SAMPLE VALUES

Serum/Plasma/Saliva/Urine/Human Milk - Samples from apparently healthy volunteers were evaluated for the presence of human ApoE in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Serum (n=36)	29,845	15,324-64,590	9524
EDTA plasma (n=36)	28,923	15,563-60,009	8804
Heparin plasma (n=36)	29,428	17,081-62,987	9473
Saliva (n=10)	9.32	3.72-16.4	3.57
Human milk (n=8)	1086	427-1523	376

Sample Type	Mean of Detectable (ng/mL)	% Detectable	Range (ng/mL)
Urine (n=10)	7.80	70	ND-15.6

ND=Non-detectable

Cell Culture Supernates:

HepG2 human hepatocellular carcinoma cells were cultured in MEM supplemented with 10% fetal bovine serum and 2 mM L-glutamine. An aliquot of the cell culture supernate was removed, assayed for human ApoE, and measured 143 ng/mL.

JE-3 human epithelial choriocarcinoma cells were cultured in DMEM supplemented with 10% fetal bovine serum. An aliquot of the cell culture supernate was removed, assayed for human ApoE, and measured 29.3 ng/mL.

SPECIFICITY

This assay recognizes natural and recombinant human Total ApoE (ApoE2, ApoE3, and ApoE4).

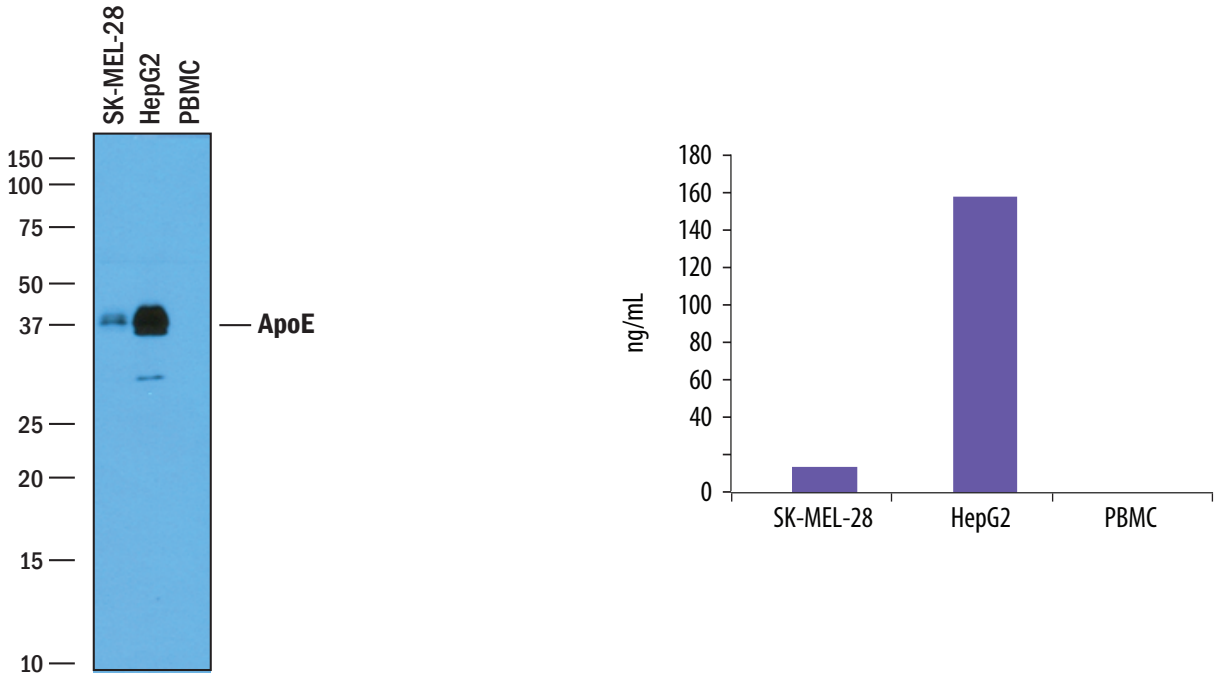
The factors listed below were prepared at 2500 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors at 2500 ng/mL in a mid-range recombinant human ApoE control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

ApoA1
ApoA2
ApoB
ApoB100
ApoC1
ApoC2
ApoD
ApoER2
ApoH
ApoM
APP770
LDL R
LRP-1 Cluster II
LRP-1 Cluster IV

Recombinant mouse:

LDL R



Cell culture media from the indicated cells was analyzed by Western blot and Quantikine ELISA. Media samples were resolved under reducing SDS-PAGE conditions, transferred to a PVDF membrane, and immunoblotted with Goat Anti-Human ApoE antibody (R&D Systems, Catalog # AF4144). The Western blot shows a direct correlation with the ELISA value for these samples.

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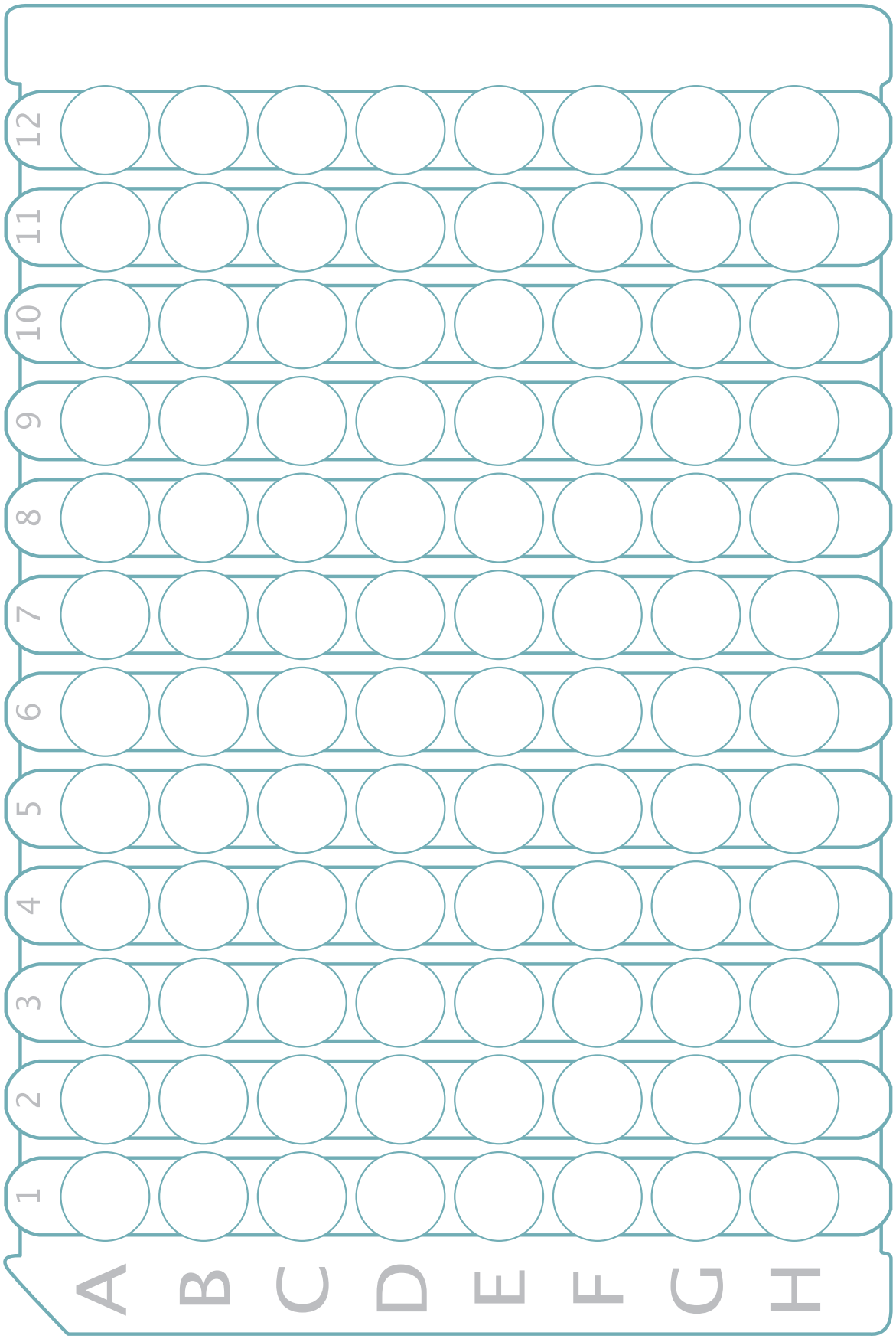
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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