ab195215 – IgG Human SimpleStep ELISA® Kit

For the quantitative measurement of human IgG in serum, plasma, milk, saliva, urine, cell culture supernatants and tissue extracts

For overview, typical data and additional information please visit: www.abcam.com/ab195215

Storage and Stability: Store kit at 2-8°C immediately upon receipt. Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Materials Supplied

Item	Quantity	Storage Condition
10X Human IgG Capture Antibody	600 µL	+4°C
10X Human IgG Detector Antibody	600 µL	+4°C
IgG Human Lyophilized Purified Protein	2 Vials	+4°C
Antibody Diluent CP2	6 mL	+4°C
10X Wash Buffer PT	20 mL	+4°C
5X Cell Extraction Buffer PTR	10 mL	+4°C
TMB Development Solution	12 mL	+4°C
Stop Solution	12 mL	+4°C
Sample Diluent NS	12 mL	+4°C
SimpleStep Pre-Coated 96-Well Microplate	96 Wells	+4°C
Plate Seal	1	+4°C

Note: Antibody Diluent CP2- This buffer has been reformulated to enhance stability after freeze-thaw cycles while producing data equivalent to the original formulation of antibody diluent CP previously used in this kit.
While we run stock down, you may receive kits containing antibody diluent CP. This does not affect the way you should use the kit.
If you have any questions please contact Abcam Scientific Support.

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

Microplate reader capable of measuring absorbance at 450 or 600 nm.
Method for determining protein concentration (BCA assay recommended).
Deionized water.
PBS (1.4 mM KH2PO4, 8 mM Na2HPO4, 140 mM NaCl, 2.7 mM KCl, pH 7.4).
Multi- and single-channel pipettes.
Tubes for standard dilution.
Plate shaker for all incubation steps.
Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).

Special Handling Instructions for the Human IgG kit

IgG can bind to the surface of the skin microbiota. To prevent unintended background, it is recommended to clean bench surfaces and all pipettes to be used during the experiment with 10% bleach. Use a surgical mask and maintain gloves clean by either using 70% ethanol or by changing them frequently. Do not leave reagents or the plate opened while working or during assay incubation.

Reagent Preparation

Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells. **The sample volumes below are sufficient for 48 wells (6 x 8-well strips); adjust volumes as needed for the number of strips in your experiment.**
Prepare only as much reagent as is needed on the day of the experiment. Capture and Detector Antibodies have only been tested for stability in the provided 10X formulations.

1X Cell Extraction Buffer PTR (For tissue extracts only) If required, prepare 1X Cell Extraction Buffer PTR by diluting 5X Cell Extraction Buffer PTR to 1X with deionized water. To make 10 mL 1X Cell Extraction Buffer PTR combine 8 mL deionized water, 2 mL 5X Cell Extraction Buffer PTR. Mix thoroughly and gently. If required protease inhibitors can be added.

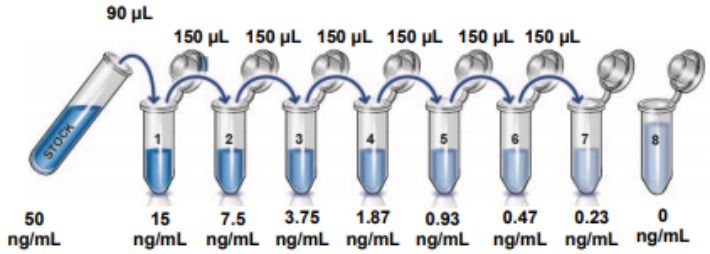
1X Wash Buffer PT: Prepare 1X Wash Buffer PT by diluting 10X Wash Buffer PT with deionized water. To make 50 mL 1X Wash Buffer PT combine 5 mL 10X Wash Buffer PT with 45 mL deionized water. Mix thoroughly and gently.

Antibody Cocktail: Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent CP2. To make 3 mL of the Antibody Cocktail combine 300 µL 10X Capture Antibody and 300 µL 10X Detector Antibody with 2.4 mL Antibody Diluent CP2. Mix thoroughly and gently.

Standard Preparation
Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of positive controls for every use. The following section describes the preparation of a standard curve for duplicate measurements (recommended).
IMPORTANT: If the protein standard vial has a volume identified on the label, reconstitute the IgG standard by adding that volume of Diluent indicated on the label. Alternatively, if the vial has a mass identified, reconstitute the IgG standard by adding 1 mL Diluent. Hold at room temperature for 10 minutes and mix gently. This is the 50 ng/mL **Stock Standard** Solution.

For serum, plasma, milk, urine, saliva and culture media measurements, reconstitute the IgG standard by adding Sample Diluent NS.
For tissue extract measurements, reconstitute the IgG standard by 1X Cell Extraction Buffer PTR.

1. Label eight tubes, Standards 1– 8.
2. Add 210 µL of appropriate sample buffer into tube number 1 and 150 µL of appropriate sample buffer into numbers 2-8.
3. Use the Stock Standard to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:



To convert sample values obtained with the kit to approximate NIBSC 67/086 units, use the following equation: NIBSC (67/086) approximate value (IU/mL) = 1.7e-5 x SimpleStep IgG value (ng/mL)

Sample Preparation

TYPICAL SAMPLE DYNAMIC RANGE	
Sample Type	**Range**
Human Serum	$1:5x10^6 – 1:8x10^7$
Human Plasma - EDTA	$1:2x10^6 – 1:3x10^7$
Human Plasma - Citrate	$1:2x10^6 – 1:3x10^7$
Human Plasma - Heparin	$1:4x10^6 – 1:6x10^7$
Human Milk	1:1,000 – 1:10,000
Human Urine	1:50 – 1:500
Human Saliva	1:1,000 – 1:10,000
Human Cerebrospinal Fluid	1:2,000 – 1:32,000

Avoid repeated freeze-thaw cycles of samples. Store Samples at -20°C or below.
Plasma Collect plasma using citrate, EDTA or heparin. Centrifuge samples at 2,000 x g for 10 minutes. Dilute samples into Sample Diluent NS and assay. Store un-diluted for up to 3 months.
Serum Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2,000 x g for 10 minutes and collect serum. Dilute samples into Sample Diluent NS and assay. Store un-diluted serum at -20°C or below.
Cell Culture Supernatants Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Collect supernatants and assay. Store samples at -20°C or below.
Urine Centrifuge urine at 2,000 x g for 10 minutes to remove debris. Collect supernatants, dilute in Sample Diluent NS and assay. Store samples at -20°C or below.
Saliva Centrifuge saliva at 800 x g for 10 minutes to remove debris. Collect supernatants dilute in Sample Diluent NS and assay. Store samples at -20°C or below.
Milk De-fat milk samples as follows. Centrifuge milk samples at 500 x g for 15 minutes at 4°C and collect the aqueous fraction using syringe attached to needle. Centrifuge the aqueous fraction at 3,000 x g for 15 minutes at 4°C and collect the final aqueous fraction (de-fatted milk) using syringe attached to needle. Dilute the de-fatted milk samples in Sample Diluent NS and assay. Store un-diluted de-fatted milk at -20°C or below.
Preparation of extracts from tissue homogenates Tissue lysates are typically prepared by homogenization of tissue that is first minced and thoroughly rinsed in PBS to remove blood (dounce homogenizer recommended). Homogenize 100 to 200 mg of wet tissue in 500 µL – 1 mL of chilled 1X Cell Extraction Buffer PTR. For lower amounts of tissue adjust volumes accordingly. Incubate on ice for 20 minutes. Centrifuge at 18,000 x g for 20 minutes at 4°C. Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay. Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.
Cerebrospinal Fluid (CSF) Dilute cerebrospinal fluid at least 1:2 into Sample Diluent NS and assay. Store un-diluted samples at -20°C or below.
Dilution of samples
Due to the high dilutions required for some samples, we recommend initially diluting your samples in 1X Wash Buffer and then performing the final dilution in Sample Diluent NS. The table below demonstrates the steps suggested to generate a final sample dilution of $1:2x10^6$. Ensure that the final dilution is equal or greater than 1:40 dilution factor to avoid a significant inadvertent dilution of the Sample Diluent NS.

Tube #	Sample to Dilute	Vol to Dilute (µL)	Vol of 1X Wash Buffer (µL)	Vol of Sample Diluent NS (µL)	Starting Conc.	Final Conc.
1	Neat	5	195	0	Neat	1:40
2	Tube #1	4	196	0	1:40	1:2,000
3	Tube #2	4	196	0	1:2,000	$1:1x10^5$
4	Tube #3	5	0	95	$1:1x10^5$	$1:2x10^6$

Plate Preparation

The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4°C.
For each assay performed, a minimum of two wells must be used as the zero control.
For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
Differences in well absorbance or "edge effects" have not been observed with this assay.

Assay Procedure

Equilibrate all materials and prepared reagents to room temperature prior to use.
We recommend that you assay all standards, controls and samples in duplicate.

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, reseal and return to 4°C storage.
3) Add 50 µL of all sample or standard to appropriate wells.
4) Add 50 µL of the Antibody Cocktail to each well.
5) Seal the plate and incubate for 40 minutes at room temperature on a plate shaker set to 400 rpm.
6) Wash each well with 3 x 350 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 350 µL 1X Wash Buffer PT into each well. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and blot it against clean paper towels to remove excess liquid.
7) Add 100 µL of TMB Development Solution to each well and incubate for 5 minutes in the dark on a plate shaker set to 400 rpm. *Given variability in laboratory environmental conditions, optimal incubation time may vary between 5 and 20 minutes.*
   Note: The addition of Stop Solution will change the color from blue to yellow and enhance the signal intensity about 3X. To avoid signal saturation, proceed to the next step before the high concentration of the standard reaches a blue color of O.D.600 equal to 1.0.
8) Add 100 µL of Stop Solution to each well. Shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm. This is an endpoint reading.
9) Alternative to 7 – 8: Instead of the endpoint reading at 450 nm, record the development of TMB Substrate kinetically. Immediately after addition of TMB Development Solution begin recording the blue color development with elapsed time in the microplate reader prepared with the following settings:

Mode	Kinetic
Wavelength	600 nm
Time	up to 20 min
Interval	20 sec - 1 min
Shake	Shake between readings

Note: that an endpoint reading can also be recorded at the completion of the kinetic read by adding 100 µL Stop Solution to each well and recording the OD at 450 nm.

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
www.abcam.com/protocols/the-complete-elisa-guide

For technical support contact information, visit: www.abcam.com/contactus

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