ab187400 – Rabbit IgG SimpleStep ELISA® Kit

For the quantitative measurement of IgG in rabbit serum, plasma and cell culture supernatants.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab187400

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 Standard Preparation and Reagent preparation sections.

Materials Supplied

Item	Quantity	Storage Condition
10X Rabbit IgG Capture Antibody	600 µL	+2-8°C
10X Rabbit IgG Detector Antibody	600 µL	+2-8°C
Rabbit IgG Lyophilized purified Protein	2 Vials	+2-8°C
Antibody Diluent 5B	6 mL	+2-8°C
10X Wash Buffer PT	20 mL	+2-8°C
TMB Development Solution	12 mL	+2-8°C
Stop Solution	12 mL	+2-8°C
Sample Diluent NS	50 mL	+2-8°C
Pre-Coated 96 Well Microplate (12 x 8 well strips)	96 Wells	+2-8°C
Plate Seal	1	+2-8°C

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.
Multi- and single-channel pipettes.
Tubes for standard dilution.
Plate shaker for all incubation steps.
Optional: Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).

Reagent Preparation

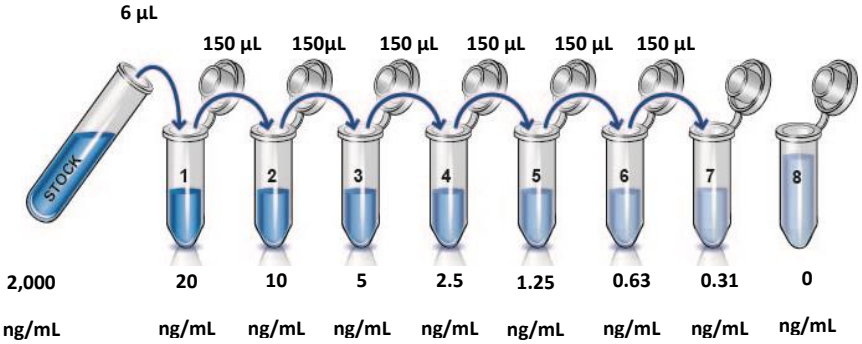
Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells. The sample volumes below are sufficient for 48 wells (6 x 8-well strips); adjust volumes as needed for the number of strips in your experiment.
Prepare only as much reagent as is needed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

1X 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 5B. Prepare the cocktail fresh immediately prior to addition to the plate. 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 5B. Mix thoroughly and gently.

Standard Preparation

IMPORTANT: If the protein standard vial has a volume identified on the label, reconstitute the rabbit IgG standard by adding that volume of Sample Diluent NS indicated on the label. Alternatively, if the vial has a mass identified, reconstitute the rabbit IgG standard by adding 1,000 µL Sample Diluent NS. Hold at room temperature for 10 minutes and mix gently. This is the 2,000 ng/mL **Stock Standard Solution**.
Label eight tubes, Standards 1– 8.
Add 594 µL Sample Diluent NS into tube number 1 and 150 µL of Sample Diluent NS into numbers 2-8.
Use the Stock Standard to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:



Sample Preparation

TYPICAL SAMPLE DYNAMIC RANGE	
Sample Type	**Range**
Normal Rabbit IgG	0.31-20 ng/mL
Normal Rabbit Serum	1:1 x 10^6 to 1:1 x 10^7
Normal Rabbit Plasma	Predicted 1:1 x 10^6 to 1:1 x 10^7
Conditioned Cell Culture Media	Predicted 1:100 -1:10,000

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 plasma samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

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. Avoid repeated freeze-thaw cycles.

Cell Culture Supernatants

Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris.
Collect supernatants and assay.
Dilute supernatants into Sample Diluent NS and assay.
Store samples at -20°C or below. Avoid repeated freeze-thaw cycles.

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 $1:1x10^6$. 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	Volume to Dilute (µL)	Volume of 1X Wash Buffer (µL)	Volume 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	20	0	180	$1:1x10^5$	$1:1x10^6$

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 1 hour 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. Wash Buffer PT should remain in wells for at least 10 seconds. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and tap gently against clean paper towels to remove excess liquid.
7. Add 100 µL of TMB Substrate to each well and incubate for 10 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
Shaking	Shake before and 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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