

## ab316264 – Mouse IgG1 Competitive SimpleStep ELISA® Kit

For the quantitative measurement of IgG1 in mouse serum, plasma (heparin), plasma (EDTA), plasma (citrate), cell culture supernatant, and urine.  
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: [www.abcam.com/ab316264](http://www.abcam.com/ab316264)

**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<br>1 x 96 tests | Quantity<br>10 x 96 tests | Storage<br>Condition |
|--|--------------------------|---------------------------|----------------------|
| Mouse IgG1 HRP Conjugate 100X            | 80 µL                    | 10 x 80 µL                | +4°C                 |
| Mouse IgG1 Lyophilized Standard          | 2 Vials                  | 10 x 2 Vials              | +4°C                 |
| Antibody Diluent 4BR                     | 8 mL                     | 10 x 8 mL                 | +4°C                 |
| Sample Diluent NS                        | 50 mL                    | 2 x 250 mL                | +4°C                 |
| Wash Buffer PT 10X                       | 20 mL                    | 200 mL                    | +4°C                 |
| TMB Development Solution                 | 12 mL                    | 120 mL                    | +4°C                 |
| Stop Solution                            | 12 mL                    | 120 mL                    | +4°C                 |
| SimpleStep Pre-Coated 96-Well Microplate | 96 wells                 | 10 x 96 wells             | +4°C                 |
| Plate Seal                               | 1                        | 10                        | +4°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.

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. The HRP Conjugates have only been tested for stability in the provided 100X formulation.

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

**HRP Conjugate Solution:** Prepare HRP Conjugate Solution by diluting the 100X HRP Conjugate in Antibody Diluent 4BR. To make 4 mL of the HRP Conjugate Solution, combine 40 µL 100X HRP Conjugate with 3.96 mL Antibody Diluent 4BR. Mix thoroughly and gently.

### Standard Preparation

Always prepare a fresh set of standards for every use. Discard working standard dilutions after use as they do not store well. The following section describes the preparation of a standard curve for duplicate measurements (recommended).

1. Reconstitute the IgG1 standard sample by adding the volume of Sample Diluent NS indicated on the standard vial label. Hold at room temperature for 10 minutes. Mix thoroughly and gently. This is the 20,000 ng/mL **Stock Standard** Solution.
2. Label eight tubes, Standards 1–8.
3. Add 360 µL of Sample Diluent NS into tube number 1 and 180 µL of Sample Diluent NS into numbers 2-8.
4. Use the **Stock Standard** to prepare the following dilution series. Standard #7 contains no protein and is the Blank control:

| Standard # | Dilution Sample       | Volume to Dilute (µL) | Volume of Diluent (µL) | Starting Conc. (ng/mL) | Final Conc. (ng/mL) |
|------------|-----------------------|-----------------------|------------------------|------------------------|---------------------|
| 1          | <b>Stock Standard</b> | 40                    | 360                    | 20,000                 | 2,000               |
| 2          | Standard#1            | 180                   | 180                    | 2,000                  | 1,000               |
| 3          | Standard#2            | 180                   | 180                    | 1,000                  | 500                 |
| 4          | Standard#3            | 180                   | 180                    | 500                    | 250                 |
| 5          | Standard#4            | 180                   | 180                    | 250                    | 125                 |
| 6          | Standard#5            | 180                   | 180                    | 125                    | 62.5                |
| 7          | Blank Control         | 0                     | 180                    | N/A                    | N/A                 |

### Sample Preparation

| Typical Sample Dynamic Range |                    |
|------------------------------|--------------------|
| Sample Type                  | Range              |
| Serum                        | 1:16,000 - 1:1,000 |
| Plasma - Heparin             | 1:8000 - 1:500     |
| Plasma - EDTA                | 1:8000 - 1:500     |
| Plasma - Citrate             | 1:8000 - 1:500     |
| Cell Culture Media           | 3.125% - 50%       |
| Urine                        | 3.125% - 50%       |

**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 at least 1:1000 into Sample Diluent NS and assay. Store un-diluted serum at -20°C or below. Avoid repeated freeze-thaw cycles.

**Plasma** Collect plasma using citrate, EDTA or heparin. Centrifuge samples at 2,000 x g for 10 minutes. Dilute samples at least 1:500 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.

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

**Urine** Centrifuge urine at 2,000 x g for 10 minutes to remove debris. Dilute samples into Sample Diluent NS and assay. Store un-diluted urine 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 final sample dilutions of 1:500. Ensure that the final dilution is equal or greater than 1:10 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             | 4                     | 196                           | 0                                | Neat           | 1:50        |
| 2      | Tube #1          | 20                    | 0                             | 180                              | 1:50           | 1:500       |

#### 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 60 µL of all sample or standard to appropriate wells.
4. Add 60 µL of the HRP Conjugate Solution 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 Development Solution 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 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](http://www.abcam.com/protocols/the-complete-elisa-guide)

**For technical support contact information, visit:** [www.abcam.com/contactus](http://www.abcam.com/contactus)

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## Additional information

### ASSAY SPECIFICITY

This kit is designed for the quantification of mouse IgG1.

The standard protein in this kit is mouse IgG1.

Native signal was detected in serum, plasma (heparin), plasma (EDTA), and plasma (citrate).

Spiked protein experiments were used to validate cell culture supernatant, and urine sample types.

Saliva, milk, CSF, cell extract, and tissue extract samples have not been tested with this kit.

### CROSS REACTIVITY

2 µg/mL of recombinant mouse IgA, IgM, and IgE, and 9 µg/mL of recombinant mouse IgG2b and IgG3 were tested for cross reactivity. No cross reactivity was observed.

### SPECIES REACTIVITY

Other species reactivity was determined by measuring 1:500 serum samples of various species, interpolating the protein concentrations from the mouse standard curve, and expressing the interpolated concentrations as a percentage of the protein concentration in mouse serum assayed at the same dilution.

No signal was observed in 1:500 serum samples from the following species: Human, Monkey, Rat, Cow

Other species reactivity not determined.

### CALCULATION

- Create a standard curve by plotting the average absorbance value for each standard concentration (y-axis) against the target concentration (x-axis) of the standard. Use graphing software to draw the best smooth curve through these points to construct the standard curve.
- Note: Most microplate reader software or graphing software will plot these values and fit a curve to the data. A four parameter curve fit (4PL) is often the best choice; however, other algorithms (e.g. linear, semi-log, log/log, 4 parameter logistic) can also be tested to determine if it provides a better curve fit to the standard values.
- Determine the concentration of the target analyte in the sample by interpolating absorbance values against the standard curve. Multiply the resulting value by the appropriate sample dilution factor, if used, to obtain the concentration of target analyte in the sample.
- Samples generating absorbance values lower than that of the highest concentration standard should be further diluted and reanalyzed. Similarly, samples which measure at an absorbance value greater than that of the lowest concentration standard should be retested in a less dilute form.
- Optional: The binding percentage, B/B<sub>0</sub>, can be calculated by dividing the average absorbance value for each standard or sample by the average absorbance of the zero standard (B<sub>0</sub>).

### TYPICAL DATA

Typical standard curve – data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

| Standard Curve Measurements |            |       |          |                      |
|-----------------------------|------------|-------|----------|----------------------|
| Concentration (ng/mL)       | O.D 450 nm |       | Mean O.D | B/B <sub>0</sub> (%) |
|                             | 1          | 2     |          |                      |
| 0 (B <sub>0</sub> )         | 2.561      | 2.527 | 2.544    | 100%                 |
| 62.5                        | 1.927      | 1.917 | 1.922    | 76%                  |
| 125                         | 1.327      | 1.336 | 1.331    | 52%                  |
| 250                         | 0.897      | 0.826 | 0.862    | 34%                  |
| 500                         | 0.519      | 0.526 | 0.522    | 21%                  |
| 1,000                       | 0.282      | 0.277 | 0.279    | 11%                  |
| 2,000                       | 0.146      | 0.151 | 0.149    | 6%                   |

Table 1. Example of mouse IgG1 standard curve in Sample Diluent NS. The IgG1 standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

### TYPICAL SAMPLE VALUES

#### Sensitivity:

The calculated minimal detectable dose (MDD) is 54.7 ng/mL. The MDD was determined by calculating the mean of zero standard replicates (n=26) and subtracting 2 standard deviations then extrapolating the corresponding concentration.

#### Recovery

Three concentrations of IgG1 were spiked in duplicate to the indicated biological matrix to evaluate signal recovery in the working range of the assay.

| Sample Type             | Average % Recovery | Range (%) |
|-------------------------|--------------------|-----------|
| 1:8000 Serum            | 106                | 100 – 114 |
| 1:8000 Plasma - Citrate | 107                | 95 – 123  |
| 1:8000 Plasma - EDTA    | 96                 | 95 – 97   |
| 1:8000 Plasma - Heparin | 96                 | 89 – 101  |
| 50% Cell culture media* | 93                 | 89 – 102  |
| 50% Urine               | 101                | 89 – 113  |

\*Media is DMEM containing 10% fetal calf serum.

## Linearity of Dilution

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Native IgG1 was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS.

| Dilution Factor | Interpolated value      | 1:1000 Mouse Serum | 1:500 Mouse Plasma (Citrate) | 1:500 Mouse Plasma (EDTA) | 1:500 Mouse Plasma (Heparin) |
|-----------------|-------------------------|--------------------|------------------------------|---------------------------|------------------------------|
| Undiluted       | ng/mL                   | 1002               | 1611                         | 1341                      | 1537                         |
|                 | <b>% Expected value</b> | 100                | 100                          | 100                       | 100                          |
| 2               | ng/mL                   | 454                | 662                          | 646                       | 727                          |
|                 | <b>% Expected value</b> | 91                 | 82                           | 96                        | 95                           |
| 4               | ng/mL                   | 252                | 318                          | 300                       | 316                          |
|                 | <b>% Expected value</b> | 101                | 79                           | 90                        | 82                           |
| 8               | ng/mL                   | 127                | 171                          | 165                       | 168                          |
|                 | <b>% Expected value</b> | 102                | 85                           | 99                        | 88                           |
| 16              | ng/mL                   | 63                 | 97                           | 74                        | 95                           |
|                 | <b>% Expected value</b> | 100                | 96                           | 88                        | 99                           |

Recombinant IgG1 was spiked in in the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS.

| Dilution Factor | Interpolated value      | 50% 10F DMEM | 50% Mouse Urine |
|-----------------|-------------------------|--------------|-----------------|
| Undiluted       | ng/mL                   | 910          | 1086            |
|                 | <b>% Expected value</b> | 100          | 100             |
| 2               | ng/mL                   | 426          | 501             |
|                 | <b>% Expected value</b> | 94           | 92              |
| 4               | ng/mL                   | 188          | 239             |
|                 | <b>% Expected value</b> | 82           | 88              |
| 8               | ng/mL                   | 122          | 137             |
|                 | <b>% Expected value</b> | 107          | 101             |
| 16              | ng/mL                   | 68           | 77              |
|                 | <b>% Expected value</b> | 120          | 113             |

## Precision

Mean coefficient of variations of interpolated values of IgG1 from three concentrations of mouse plasma (citrate) within the working range of the assay.

|               | Intra-assay | Inter-assay |
|---------------|-------------|-------------|
| <b>N=</b>     | 8           | 3           |
| <b>CV (%)</b> | 9.83        | 8.93        |

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