# ab195215 – Human IgG SimpleStep ELISA® Kit

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

**Note:** Sample incubation time has changed to 60 minutes.

This kit is available in a 384-well plate format. This plate utilises smaller volumes of standards and samples per well. Directions for using this format can be found on pages 7-8.

**Note:** This kit is designed to detect total human IgG across all four isotypes and uses a natively purified human IgG standard. Some variability may be observed due to differences in sample isotype composition and the native standard material. For isotype-specific measurement, we offer the kits below:
- Human IgG1: ab317543
- Human IgG2: ab202402
- Human IgG3: ab318929
- Human IgG4: ab320788

## Special Handling Instructions

This assay is sensitive to contamination from exogenous human IgG, which may increase background signal and affect assay accuracy. Human IgG is present at high levels on skin and in saliva and may be introduced during assay performance; therefore, precautions must be taken to minimize contamination. Prior to initiating the assay, bench surfaces and all pipettes to be used during the procedure should be cleaned with 10% bleach. Wear a surgical mask throughout the procedure and maintain clean gloves by frequent replacement or treatment with 70% ethanol. Reagents and the assay plate should not be left uncovered during assay setup or incubation periods, to minimize the risk of contamination.

**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.

**Directions for 96-well Plate format:**

**Materials Supplied for 96-well Format**

| Item | Quantity 96 tests | Quantiy 10 X 96 tests | Storage Condition |
|---|---|---|---|
| Human IgG Capture Antibody 10X | 600 µL | 10 X 600 µL | +4°C |
| Human IgG Detector Antibody 10X | 600 µL | 10 X 600 µL | +4°C |
| Human IgG Lyophilized Purified Protein | 2 Vials | 20 Vials | +4°C |
| Antibody Diluent CP2 | 6 mL | 10 X 6 mL | +4°C |
| Cell Extraction Buffer PTR 5X | 10 mL | 2 X 50 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:

**Because this assay is sensitive to exogenous human IgG contamination, the use of clean PPE with a surgical mask, decontaminated work surfaces/equipment, and fresh aerosol-resistant tips is strongly recommended.**

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).

## Reagent Preparation

**To minimize contamination, prepare reagents in a clean area using freshly cleaned pipettes and fresh aerosol-resistant tips. Keep reagent containers closed when not in use and avoid prolonged exposure of open tubes to the laboratory environment.**

Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for one full plate. The sample volumes below are sufficient for 48 wells (6 x 8-well strips) in the 96-well format; 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 cell and tissue extracts only):** Prepare 1X Cell Extraction Buffer PTR by diluting Cell Extraction Buffer PTR 5X to 1X with deionized water. To make 10 mL 1X Cell Extraction Buffer PTR combine 8 mL deionized water and 2 mL Cell Extraction Buffer PTR 5X. Mix thoroughly and gently. If required protease inhibitors can be added.

**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.

**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

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).

**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, saliva, urine, CSF, and cell culture supernatant **samples measurements**, use Sample Diluent NS. For tissue extract **samples measurements**, use 1X Cell Extraction Buffer PTR.

1. Label eight tubes, Standards 1– 8.
2. Use the same Sample Diluent as used to resuspend the Stock Standard to prepare the standard curve. Add 210 µL of Sample Diluent into tube number 1 and 150 µL of Sample Diluent 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:

| Standard # | Dilution Sample | Volume to Dilute (µL) | Volume of Diluent (µL) | Starting Conc. (ng/mL) | Final Conc. (ng/mL) |
|---|---|---|---|---|---|
| 1 | **Stock Standard** | 90 | 210 | 50 | 15 |
| 2 | Standard#1 | 150 | 150 | 15 | 7.5 |
| 3 | Standard#2 | 150 | 150 | 7.5 | 3.75 |
| 4 | Standard#3 | 150 | 150 | 3.75 | 1.87 |
| 5 | Standard#4 | 150 | 150 | 1.87 | 0.93 |
| 6 | Standard#5 | 150 | 150 | 0.93 | 0.47 |
| 7 | Standard#6 | 150 | 150 | 0.47 | 0.23 |
| 8 | Blank Control | 0 | 150 | N/A | N/A |

**Elevated blank signal or abnormal standard curve shape may indicate environmental or operator-derived contamination.**

## 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 |
| Cell Culture Media | ≤ 100% |
| Human Cerebrospinal Fluid | 1:2,000 – 1:32,000 |
| Human Liver Extract | 12.5 – 200 ng/mL |

**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 | 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 | 8 | 192 | 0 | 1:2,000 | $1:5x10^4$ |
| 4 | Tube #3 | 5 | 0 | 195 | $1:5x10^4$ | $1:2x10^6$ |

**For sample types expected to contain high endogenous human IgG, accurate dilution is critical. Results outside the expected dilution range, unusually high background, variability of replicates, or poor dilution linearity may indicate insufficient dilution and/or contamination during handling.**

**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 $1: 5x10^6$ 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 plasma (EDTA or citrate) samples at least $1:2x10^6$ into Sample Diluent NS and assay. Dilute plasma (heparin) samples at least $1:4x10^6$ 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.

**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 at least 1:1,000 into Sample Diluent NS

**Urine** Centrifuge urine at 2,000 x g for 10 minutes to remove debris. Dilute samples at least 1:50 into Sample Diluent NS and assay. Store un-diluted urine samples at -20°C or below. Avoid repeated freeze-thaw cycles.

**Saliva** Centrifuge saliva at 800 x g for 10 minutes to remove debris. Collect supernatants and dilute samples at least 1:1,000 into Sample Diluent NS and assay. Store un-diluted samples 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. Or dilute samples into Sample Diluent NS and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

**Cerebrospinal Fluid (CSF)** Dilute cerebrospinal fluid at least 1:2,000 into Sample Diluent NS and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

**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.

## Plate Preparation

**To reduce contamination risk, open the plate only when ready to add reagents, minimize plate exposure to the environment, and reseal promptly during incubations.**

The 96 well plate strips or 384-well plate 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 for 96-well Plate Format

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 promptly after reagent addition to minimize environmental contamination 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

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# ab195215 – Human IgG SimpleStep ELISA® Kit

## Additional information

### ASSAY SPECIFICITY

This kit is designed for the quantification of Human IgG and is intended to detect all four human IgG isotypes (IgG1, IgG2, IgG3, and IgG4).

The standard protein in this kit is a natively purified Human IgG.

Due to differences in isotype composition and antibody affinity for the individual IgG isotypes, some variability in assay response may be observed between samples or lots. For applications requiring measurement of individual IgG isotypes, please refer to the isotype-specific kits listed at the beginning of this booklet.

Native signal was detected in serum, plasma (heparin), plasma (EDTA), plasma (citrate), saliva, urine, milk, CSF, and tissue extract.

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

Cell extract samples have not been tested with this kit.

### CROSS REACTIVITY

10 ng/mL of purified Human IgA, 10 ng/mL of purified Human IgM and 10 ng/mL of purified Human IgE were tested for cross reactivity. No cross reactivity was observed.

### INTERFERENCE

250 ng/mL of purified Human IgM target and 250 ng/mL of purified Human IgE were tested for interference with 5 ng/mL of purified Human IgG. No interference was observed.

250 ng/mL of purified Human IgA increased interpolated value of 5 ng/mL of purified Human IgG by 11%.

### SPECIES REACTIVITY

10 ng/mL of purified Mouse, Rat, Rabbit, Canine, Guinea, Bovine, Goat and Sheep IgG were tested for reactivity. No reactivity was observed.

Other species reactivity not determined.

### CALCULATION

- Calculate the average absorbance value for the blank control (zero) standards. Subtract the average blank control standard absorbance value from all other absorbance values.
- Create a standard curve by plotting the average blank control subtracted absorbance value for each standard concentration (y-axis) against the target protein concentration (x-axis) of the standard. Use graphing software to draw the best smooth curve through these points to construct the standard curve.

  Δ 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 protein in the sample by interpolating the blank control subtracted absorbance values against the standard curve. Multiply the resulting value by the appropriate sample dilution factor, if used, to obtain the concentration of target protein in the sample.
- Samples generating absorbance values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at an absorbance value less than that of the lowest standard should be retested in a less dilute form.

### CALIBRATION

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)

### 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 |
| | 1 | 2 | |
| 0 | 0.07 | 0.07 | 0.07 |
| 0.23 | 0.13 | 0.12 | 0.13 |
| 0.46 | 0.18 | 0.16 | 0.17 |
| 0.93 | 0.26 | 0.23 | 0.25 |
| 1.87 | 0.45 | 0.28 | 0.42 |
| 3.75 | 0.79 | 0.75 | 0.77 |
| 7.5 | 1.44 | 1.30 | 1.37 |
| 15 | 2.45 | 2.26 | 2.35 |

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

| Standard Curve Measurements | | | |
|---|---|---|---|
| Concentration (ng/mL) | O.D 450 nm | | Mean O.D |
| | 1 | 2 | |
| 0 | 0.081 | 0.083 | 0.082 |
| 0.23 | 0.14 | 0.13 | 0.13 |
| 0.46 | 0.17 | 0.17 | 0.17 |
| 0.93 | 0.26 | 0.25 | 0.25 |
| 1.87 | 0.38 | 0.39 | 0.38 |
| 3.75 | 0.69 | 0.67 | 0.68 |
| 7.5 | 1.14 | 1.18 | 1.16 |
| 15 | 1.79 | 1.96 | 1.87 |

Table 1. Example of Human IgG standard curve in 1X Cell Extraction Buffer PTR. The IgG standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

## TYPICAL SAMPLE VALUES

Performance characteristics described in this booklet were established under controlled handling conditions designed to minimize exogenous human IgG contamination. Results obtained under different laboratory handling conditions may vary.

### Sensitivity:

The calculated minimal detectable dose (MDD) is 20 pg/mL. The MDD was determined by calculating the mean of zero standard replicates (n=24) and adding 2 standard deviations then extrapolating the corresponding concentration.

### Recovery

Three concentrations of IgG 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 (%) |
|---|---|---|
| 1:2x10$^7$ Human Serum | 101 | 88 - 125 |
| 1:4x10$^6$ Human Plasma – EDTA | 90 | 87 - 93 |
| 1:4x10$^6$ Human Plasma – Citrate | 100 | 98 - 102 |
| 1:8x10$^6$ Human Plasma – Heparin | 100 | 100 - 100 |
| 1:80,000 Human Milk | 89 | 83 - 94 |
| 1:4,000 Human Urine | 87 | 82 - 93 |
| 1:40,000 Human Saliva | 89 | 80 - 106 |
| 1:4,000 Human Cerebral Spinal Fluid | 98 | 88 - 105 |
| 1:10 Cell Culture Media* | 107 | 96 - 115 |

*Media is RPMI 1640 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 IgG was measured in the following biological samples in a 2-fold dilution series. Recombinant IgG was spiked into the cell culture media in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS.

| Dilution Factor | Interpolated value | 1:5x10$^6$ Human Serum | 1:2x10$^6$ Human Plasma (Citrate) | 1:2x10$^6$ Human Plasma (EDTA) | 1:4x10$^6$ Human Plasma (Heparin) | 1:10 Culture Media |
|---|---|---|---|---|---|---|
| Undiluted | ng/mL | 3.46 | 7.02 | 5.88 | 4.59 | 8.35 |
| | **% Expected value** | 100 | 100 | 100 | 100 | 100 |
| 2 | ng/mL | 1.86 | 3.37 | 2.92 | 2.17 | 4.03 |
| | **% Expected value** | 105 | 96 | 99 | 95 | 96 |
| 4 | ng/mL | 0.86 | 1.69 | 1.54 | 1.14 | 1.77 |
| | **% Expected value** | 99 | 96 | 105 | 99 | 86 |
| 8 | ng/mL | 0.47 | 0.84 | 0.73 | 0.52 | 1.01 |
| | **% Expected value** | 108 | 96 | 99 | 91 | 97 |
| 16 | ng/mL | 0.23 | 0.40 | 0.39 | 0.24 | 0.56 |
| | **% Expected value** | 105 | 90 | 105 | 85 | 107 |

| Dilution Factor | Interpolated value | 1:1,000 Human Milk | 1:50 Human Urine | 1:1,000 Human Saliva | 1:2,000 Human CSF | 200 ng/mL Human Liver Tissue Extract |
|---|---|---|---|---|---|---|
| Undiluted | ng/mL | 16.9 | 16.65 | 17.69 | 9.40 | 19.16 |
| | **% Expected value** | 100 | 100 | 100 | 100 | 100 |
| 2 | ng/mL | 7.21 | 7.88 | 9.74 | 4.62 | 9.89 |
| | **% Expected value** | 85 | 95 | 108 | 98 | 103 |
| 4 | ng/mL | 4.6 | 4.71 | 5.04 | 2.09 | 4.82 |
| | **% Expected value** | 109 | 113 | 112 | 89 | 101 |
| 8 | ng/mL | 2.26 | 2.31 | 2.45 | 1.16 | 2.28 |
| | **% Expected value** | 107 | 111 | 109 | 98 | 95 |
| 16 | ng/mL | 1.14 | 1.21 | 1.32 | 0.58 | 1.16 |
| | **% Expected value** | 108 | 117 | 117 | 98 | 97 |

## Precision

Mean coefficient of variations of interpolated values of IgG from three concentrations of human serum within the working range of the assay.

| | Intra-assay | Inter-assay |
|---|---|---|
| **N=** | 8 | 3 |
| **CV (%)** | 6.4 | 14.7 |

**Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:**

www.abcam.com/protocols/the-complete-elisa-guide

## Technical Support

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## Directions for 384-well Plate format:

### Materials Supplied for 384-well format

| Item | Quantity | Storage Condition |
|---|---|---|
| Human IgG Capture Antibody 10X | 600 µL | +4°C |
| Human IgG Detector Antibody 10X | 600 µL | +4°C |
| Human IgG Lyophilized Recombinant Protein | 2 Vials | +4°C |
| Antibody Diluent CP2 | 6 mL | +4°C |
| Cell Extraction Buffer PTR 5X | 50 mL | +4°C |
| Sample Diluent NS | 2 x 50 mL | +4°C |
| Wash Buffer PT 10X | 2 x 20 mL | +4°C |
| TMB Development Solution | 2 x12 mL | +4°C |
| Stop Solution | 2 x12 mL | +4°C |
| SimpleStep Pre-Coated 384-Well Microplate | 384 wells | +4°C |
| Plate Seal | 1 | +4°C |

### Materials Required, Not Supplied

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

**Because this assay is sensitive to exogenous human IgG contamination, the use of clean PPE, decontaminated work surfaces/equipment, and fresh aerosol-resistant tips is strongly recommended.**

Microplate reader capable of measuring absorbance at 450 or 600 nm in a 384-well plate.
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).
Optional: Automated liquid handler.

### Reagent Preparation

**To minimize contamination, prepare reagents in a clean area using freshly cleaned pipettes and fresh aerosol-resistant tips. Keep reagent containers closed when not in use and avoid prolonged exposure of open tubes to the laboratory environment.**

Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for one full plate. The sample volumes below are sufficient for running all 384 wells; adjust volumes as needed for the number of samples and dilution scheme for 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 cell and tissue extracts only):** Prepare 1X Cell Extraction Buffer PTR by diluting Cell Extraction Buffer PTR 5X to 1X with deionized water. To make 250 mL 1X Cell Extraction Buffer PTR combine 200 mL deionized water and 50 mL Cell Extraction Buffer PTR 5X. Mix thoroughly and gently. If required protease inhibitors can be added.

**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.

**Antibody Cocktail:** Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent CP2. To make 6 mL of the Antibody Cocktail combine 600 µL 10X Capture Antibody and 600 µL 10X Detector Antibody with 4.8 mL Antibody Diluent CP2. 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).

**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, saliva, urine, CSF, and cell culture supernatant **samples measurements**, use Sample Diluent NS. For tissue extract **samples measurements**, use 1X Cell Extraction Buffer PTR.

1. Label eight tubes, Standards 1– 8.
2. Use the same Sample Diluent as used to resuspend the Stock Standard to prepare the standard curve. Add 105 µL of Sample Diluent into tube number 1 and 75 µL of Sample Diluent 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:

| Standard # | Dilution Sample | Volume to Dilute (µL) | Volume of Diluent (µL) | Starting Conc. (ng/mL) | Final Conc. (ng/mL) |
|---|---|---|---|---|---|
| 1 | **Stock Standard** | 45 | 105 | 50 | 15 |
| 2 | Standard#1 | 75 | 75 | 15 | 7.5 |
| 3 | Standard#2 | 75 | 75 | 7.5 | 3.75 |
| 4 | Standard#3 | 75 | 75 | 3.75 | 1.87 |
| 5 | Standard#4 | 75 | 75 | 1.87 | 0.93 |
| 6 | Standard#5 | 75 | 75 | 0.93 | 0.47 |
| 7 | Standard#6 | 75 | 75 | 0.47 | 0.23 |
| 8 | Blank Control | 0 | 75 | N/A | N/A |

**Elevated blank signal or abnormal standard curve shape may indicate environmental or operator-derived contamination.**

## Plate Preparation

**To reduce contamination risk, open the plate only when ready to add reagents, minimize plate exposure to the environment, and reseal promptly during incubations.**

The 384-well plate included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
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 for 384-well Plate Format

**The 384-well format uses smaller volumes and may be more sensitive to contamination introduced during setup; strict contamination-control practices are recommended.**

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. Add 12.5 µL of all sample or standard to appropriate wells.
3. Add 12.5 µL of the Antibody Cocktail to each well.
4. Seal the plate and incubate for **1 hour** at room temperature on a plate shaker set to 700 rpm.
5. Wash each well with 3 x 100 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 100 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.
6. Add 25 µL of TMB Development Solution to each well and incubate for 10 minutes in the dark on a plate shaker set to 700 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.
7. Add 25 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. Proper mixing of the Stop Solution is required for proper measurement.
8. Alternative to 6 – 7: 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 25 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

## Technical Support

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