ab317776 – Human IgE SimpleStep ELISA® Kit

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

For overview, typical data and additional information please visit: www.abcam.com/ab317776

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 5 and 6.

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 1 x 96 tests	Quantity 10 x 96 tests	Storage Condition
Human IgE Capture Antibody 10X	600 µL	10 x 600 µL	+4°C
Human IgE Detector Antibody 10X	600 µL	10 x 600 µL	+4°C
Human IgE Lyophilized Recombinant Protein	2 Vials	10 x 2 Vials	+4°C
Antibody Diluent 5BR	6 mL	10 x 6 mL	+4°C
Cell Extraction Enhancer Solution 50X	1 mL	10 x 1 mL	+4°C
Denaturant	500 µL	10 x 500 µL	+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.
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.

The provided Cell Extraction Enhancer Solution 50X may precipitate when stored at + 4°C. To dissolve, warm briefly at + 37°C and mix gently. The Cell Extraction Enhancer Solution 50X can be stored at room temperature to avoid precipitation.

Sample Diluent NS + 1X Enhancer: Prepare Sample Diluent NS + 1X Enhancer by combining Sample Diluent NS and 50X Cell Extraction Enhancer Solution. To make 5 mL Sample Diluent NS + 1X Enhancer, combine 4.9 mL Sample Diluent NS and 100 µL Cell Extraction Enhancer Solution 50X. Mix thoroughly and gently.

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 5BR. 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 5BR. Mix thoroughly and gently.

2X Denaturant: Prepare 2X Denaturant by combining Sample Diluent NS and Denaturant. To make 900 µL of 2X Denaturant combine 100 µL of Denaturant with 800 µL of Sample Diluent NS. 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 IgE standard sample by adding the volume of Sample Diluent NS + 1X Enhancer indicated on the protein vial label. Hold at room temperature for 10 minutes. Mix thoroughly and gently. This is the 50,000 pg/mL **Stock Standard** Solution.
2. Label eight tubes, Standards 1– 8.
3. Add 344 µL of Sample Diluent NS + 1X Enhancer into tube number 1 and 150 µL of Sample Diluent NS + 1X Enhancer into numbers 2-8.
4. 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. (pg/mL)	Final Conc. (pg/mL)
1	**Stock Standard**	56	344	50,000	7,000
2	Standard#1	150	150	7,000	3,500
3	Standard#2	150	150	3,500	1,750
4	Standard#3	150	150	1,750	875
5	Standard#4	150	150	875	437.5
6	Standard#5	150	150	437.5	218.75
7	Standard#6	150	150	218.75	109.38
8	Blank Control	0	150	0	0

Sample Preparation

Typical Sample Dynamic Range	
Sample Type	**Range**
Serum	1:640 – 1:40
Plasma – Citrate	1:640 – 1:40
Plasma – EDTA	1:640 – 1:40
Cell Culture Media	≤50%
Urine	≤50%
Saliva	≤25%

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. Store un-diluted serum at -20°C or below. Avoid repeated freeze-thaw cycles.

1. To one volume part of neat serum add one volume part of 2X Denaturant.
2. Incubate samples at room temperature for 10 minutes.
3. Dilute samples 20-fold in sample diluent NS, for a final concentration of 1:40.
4. Assay, or dilute samples into Sample Diluent NS + 1X Enhancer and assay.

Plasma Collect plasma using citrate or EDTA. Centrifuge samples at 2,000 x g for 10 minutes. Store un-diluted plasma samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles. Note: This kit is incompatible with plasma (heparin) samples.

1. To one volume part of neat plasma add one volume part of 2X Denaturant.
2. Incubate samples at room temperature for 10 minutes.
3. Dilute samples 20-fold in sample diluent NS, for a final concentration of 1:40.
4. Assay, or dilute samples into Sample Diluent NS + 1X Enhancer and assay.

Dilution Scheme For Serum and Plasma Samples:

Tube #	Sample to Dilute	Volume to Dilute (µL)	Volume of 2X Denaturant	Volume of Sample Diluent NS	Starting Dilution	Final Dilution
1	**Neat Serum or Plasma**	20	20	0	Neat	1:2
2	Tube #1	20	0	380	1:2	1:40

Cell Culture Supernatants Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Collect supernatants Dilute samples at least 1:2 into Sample Diluent NS + 1X Enhancer 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 at least 1:2 into Sample Diluent NS + 1X Enhancer 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. Dilute samples at least 1:4 into Sample Diluent NS + 1X Enhancer and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

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 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

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

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Additional information

ASSAY SPECIFICITY
This kit is designed for the quantification of human IgE.

The standard protein in this kit is the full-length isoform 1 of human Immunoglobulin heavy constant epsilon (Uniprot P01854-1).

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

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

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

This kit is incompatible with plasma (heparin) samples.

CROSS REACTIVITY
50 ng/mL of Human IgG1, IgG2, IgG3, IgG4, IgA, IgD, and IgM were tested for cross reactivity. No cross reactivity was observed.

INTERFERENCE
50 ng/mL of Human IgG1, IgG2, IgG3, IgG4, IgA, IgD, and IgM were tested for interference with 1.75 ng/mL of recombinant Human IgE. No interference was observed.

SPECIES REACTIVITY
Other species reactivity was determined by measuring 1:40 serum samples of various species, interpolating the protein concentrations from the human standard curve, and expressing the interpolated concentrations as a percentage of the protein concentration in human serum assayed at the same dilution.

No signal was observed for the following species: Mouse, Rat, Cow.

Reactivity was determined for the following species: Monkey (100%).

Other species reactivity not determined.

CALIBRATION
The NIBSC/WHO unclassified purified human IgE preparation 11/234 was evaluated in this kit. The dose response curve of the unclassified standard IgE parallels the SimpleStep standard curve. To convert sample values obtained with the SimpleStep Human IgE kit to approximate NIBSC (11/234) units, use the equation below.

NIBSC (11/234) approximate value (IU/mL) = 0.0007 x SimpleStep human IgE value (pg/mL).

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 absorbance values less than that of the lowest standard should be retested in a less dilute form.

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 (pg/mL)	**O.D 450 nm**		**Mean O.D**
	1	**2**	
0	0.042	0.046	0.044
109.38	0.097	0.098	0.097
218.75	0.145	0.148	0.147
437.5	0.259	0.253	0.256
875	0.459	0.474	0.467
1,750	0.848	0.840	0.844
3,500	1.610	1.643	1.627
7,000	3.014	2.953	2.984

Table 1. Example of human IgE standard curve in Sample Diluent NS + 1X Enhancer. The IgE 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 23.1 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 IgE 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 (%)
1:100 Serum	81	77 - 84
1:100 Plasma – Citrate	97	89 - 101
1:100 Plasma – EDTA	85	80 - 88
50% Cell Culture Media*	93	90 - 94
50% Urine	100	98 - 103
25% Saliva	97	93 - 99

*Media is DMEM containing 10% fetal bovine 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 IgE was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS + 1X Enhancer.

Dilution Factor	Interpolated value	1:40 Human Serum	1:40 Human Plasma (Citrate)	1:40 Human Plasma (EDTA)
Undiluted	pg/mL	6,094	4,156	5,789
	% Expected value	100	100	100
2	pg/mL	3,012	2128	2,999
	% Expected value	99	102	104
4	pg/mL	1,457	1,097	1,512
	% Expected value	96	106	104
8	pg/mL	729.5	528.2	732.8
	% Expected value	96	102	101
16	pg/mL	383.2	280.6	366.8
	% Expected value	101	108	101

Recombinant IgE was spiked into the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS + 1X Enhancer.

Dilution Factor	Interpolated value	50% Cell Culture Media	50% Urine	25% Saliva
Undiluted	pg/mL	3,303	3,496	3,235
	% Expected value	100	100	100
2	pg/mL	1,695	1,796	1,694
	% Expected value	103	103	105
4	pg/mL	880.4	928.1	856.4
	% Expected value	107	106	106
8	pg/mL	439.6	441.7	435
	% Expected value	106	101	108
16	pg/mL	240.8	218	219
	% Expected value	117	100	108

Precision

Mean coefficient of variations of interpolated values of IgE from a single concentration of serum within the working range of the assay.

	Intra-assay	Inter-assay
N=	8	3
CV (%)	2.4	8.6

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

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DIRECTIONS FOR 384-WELL PLATE FORMAT:

Materials Supplied for 384-well Format

Item	Quantity	Storage Condition
Human IgE Capture Antibody 10X	600 µL	+4°C
Human IgE Detector Antibody 10X	600 µL	+4°C
Human IgE Lyophilized Recombinant Protein	2 Vials	+4°C
Antibody Diluent 5BR	6 mL	+4°C
Cell Extraction Enhancer Solution 50X	5 mL	+4°C
Denaturant	500 µL	+4°C
Sample Diluent NS	2 x 50 mL	+4°C
Wash Buffer PT 10X	20 mL	+4°C
TMB Development Solution	2 x 12 mL	+4°C
Stop Solution	2 x 12 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:

Microplate reader capable of measuring absorbance at 450 or 600 nm in a 384-well plate.
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

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.

The provided Cell Extraction Enhancer Solution 50X may precipitate when stored at + 4°C. To dissolve, warm briefly at + 37°C and mix gently. The Cell Extraction Enhancer Solution 50X can be stored at room temperature to avoid precipitation.

Sample Diluent NS + 1X Enhancer: Prepare Sample Diluent NS + 1X Enhancer by combining Sample Diluent NS and 50X Cell Extraction Enhancer Solution. To make 50 mL Sample Diluent NS + 1X Enhancer, combine 49 mL Sample Diluent NS and 1 mL Cell Extraction Enhancer Solution 50X. Mix thoroughly and gently.

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 5BR. 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 5BR. Mix thoroughly and gently.

2X Denaturant: Prepare 2X Denaturant by combining Sample Diluent NS and Denaturant. To make 9 mL of 2X Denaturant combine 1 mL of Denaturant with 8 mL of Sample Diluent NS. 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 IgE standard sample by adding the volume of Sample Diluent NS + 1X Enhancer indicated on the protein vial label. Hold at room temperature for 10 minutes. Mix thoroughly and gently. This is the 50,000 pg/mL **Stock Standard** Solution.
2. Label eight tubes, Standards 1– 8.
3. Add 172 µL of Sample Diluent NS + 1X Enhancer into tube number 1 and 75 µL of Sample Diluent NS + 1X Enhancer into numbers 2-8.
4. 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. (pg/mL)	Final Conc. (pg/mL)
1	**Stock Standard**	28	172	50,000	7,000
2	Standard#1	75	75	7,000	3,500
3	Standard#2	75	75	3,500	1,750
4	Standard#3	75	75	1,750	875
5	Standard#4	75	75	875	437.5
6	Standard#5	75	75	437.5	218.75
7	Standard#6	75	75	218.75	109.38
8	Blank Control	0	75	0	0

Plate Preparation

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

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:
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