

ab327286 – Chicken Immunoglobulin Y SimpleStep ELISA® Kit (IgY), Chemiluminescent

For the quantitative measurement of Immunoglobulin Y in chicken serum, plasma (citrate), plasma (EDTA), plasma (heparin), chicken egg yolk, and mayonnaise.
For research use only - not intended for diagnostic use.
Patent pending.

For overview, typical data and additional information please visit: www.abcam.com/ab327286

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.

Limitations: All data, except typical standard curve and sensitivity were collected using the colorimetric version of this kit (ab189577).

Materials Supplied

Item	Quantity 1 x 96 tests	Quantity 10 x 96 tests	Storage Condition
Chicken Immunoglobulin Y Capture Antibody 10X	600 µL	10 x 600 µL	+4°C
Chicken Immunoglobulin Y Detector Antibody 10X	600 µL	10 x 600 µL	+4°C
Chicken Immunoglobulin Y Lyophilized Native Protein	2 Vials	10 x 2 Vials	+4°C
Antibody Diluent 4B	6 mL	10 x 6 mL	+4°C
Cell Extraction Buffer PTR 5X	10 mL	2 x 50 mL	+4°C
Cell Extraction Enhancer Solution 50X	1 mL	10 x 1 mL	+4°C
Sample Diluent NS	50 mL	2 x 250 mL	+4°C
Wash Buffer PT 10X	20 mL	200 mL	+4°C
ChemiHRP Reagent A	3 mL	50 mL	+4°C
ChemiHRP Reagent B	3 mL	50 mL	+4°C
SimpleStep Pre-Coated Black 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:

Luminometer capable of reading strip plates with the following settings: 0.5-1 second/well read time; summation mode (all wavelengths).

Method for determining protein concentration (BCA assay recommended).

Deionized water.

Multi- and single-channel pipettes.

Tubes for standard dilution.

Orbital microplate shaker for all incubation steps: capable of 750 rpm shaking speed.

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.

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.

1X Cell Extraction Buffer PTR : 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 7.8 mL deionized water, 2 mL Cell Extraction Buffer PTR 5X, and 200 µL 50X Cell Extraction Enhancer Solution. Mix thoroughly and gently. If required protease inhibitors can be added.

Antibody Cocktail: Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent 4B. 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 4B. Mix thoroughly and gently.

Lumi HRP Development Solution: Just prior to use, prepare Lumi HRP Development Solution by mixing equal volume of the ChemiHRP Reagent A and the ChemiHRP Reagent B. To make 3 mL of the Lumi HRP Development Solution combine 1.5 mL of ChemiHRP Reagent A and 1.5 mL of ChemiHRP Reagent B. Mix thoroughly and gently by inversion or slow pipetting (Avoid shaking or vortexing). Protect the prepared solution from light until use.

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 Immunoglobulin Y standard sample by adding the volume of Sample Diluent NS indicated on the protein vial label. Hold at room temperature for 10 minutes. Mix thoroughly and gently. This is the 2,000 ng/mL **Stock Standard** Solution.
- 2. Label nine tubes, Standards 1– 9.
- 3. Add 384 µL of Sample Diluent NS into tube number 1 and 150 µL of Sample Diluent NS into numbers 2-9.
- 4. Use the **Stock Standard** to prepare the following dilution series. Standard #9 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	16	384	2,000,000	80,000
2	Standard#1	75	150	80,000	26,667
3	Standard#2	75	150	26,667	8,889
4	Standard#3	75	150	8,889	2,963
5	Standard#4	75	150	2,963	988
6	Standard#5	75	150	988	329
7	Standard#6	75	150	329	110
8	Standard#7	75	150	110	36.6
9	Blank Control	0	150	0	0

Sample Preparation

Typical Sample Dynamic Range	
Sample Type	Range
Serum	1: 100 million - 1: 1 million
Plasma – Citrate*	1: 100 million - 1: 1 million
Plasma – EDTA*	1: 100 million - 1: 1 million
Plasma – Heparin*	1: 100 million - 1: 1 million
Egg Yolk*	1: 100 million - 1: 100,000
Mayonnaise	1:80,000 – 1:10,000

*Predicted dynamic range

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 1:10,000 into 1X Wash Buffer, then further dilute 100-fold 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 1:10,000 into 1X Wash Buffer, then further dilute 100-fold 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.

Mayonnaise Dilute samples at least 1:10,000 into Sample Diluent NS and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

Egg Yolk Egg yolk should be collected from a fresh egg and separated from the egg white using yolk separators. Add 2 volumes of cold (4°C) 1X Cell Extraction Buffer PTR to 1 volume of egg yolk and homogenize for 1 minute using a blender at high speed. Centrifuge sample at 16,000 x g for 10 minutes at 4°C. Collect supernatant for assay. The egg yolk lysate needs to be pre-diluted 1:1,000 into 1X Cell Extraction Buffer PTR. Sample needs to be further diluted to the working range into Sample Diluent NS. Store un-diluted egg yolk lysate 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 30 minutes at room temperature on a plate shaker set to 750 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 30 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 50 µL of prepared Lumi HRP Development Solution to each well and incubate for 1 minute in the dark on a plate shaker set to 750 rpm. Further optimization of incubation time vs signal strength can be performed if needed. Avoid introducing bubbles into the wells.
8. Measure the produced light of each well using a microplate luminometer with the following settings: 0.5-1 second/well read time in summation mode (all wavelengths). Relative light unit (RLU) readings may vary between luminometer models. It is recommended to configure instrument settings according to the manufacturer's specifications. Note: Relative light unit (RLU) values may change over the course of the 15-minute reading window.
9. Analyze the data as described below.

Mode:	Luminescence
Instrument settings:	Endpoint
Detection Mode:	All wavelengths
Read Time:	0.5-1 sec
Read:	Top

Note For microplate readers with Pre-Read Optimization option, the Read Height as well as Microplate Optimization is recommended before the first read.

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
<https://www.abcam.com/en-us/technical-resources/guides/elisa-guide>

Technical Support

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Additional information

ASSAY SPECIFICITY

This kit is designed for the quantification of chicken Immunoglobulin Y.

The standard protein in this kit is native chicken Immunoglobulin Y.

Native signal was detected in serum, chicken egg, and mayonnaise samples.

SPECIES REACTIVITY

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

Reactivity < 0.04% was determined for the following species:

- Mouse
- Rat
- Human
- Cow
- Rabbit
- Guinea Pig
- Hamster
- Pig
- Dog
- Goat

Other species reactivity not determined.

CALCULATION

- Preconfigured protocols are available when using SoftMax Pro software from Molecular Devices.
- Calculate the average chemiluminescence value for the blank control (zero) standards. Subtract the average blank control standard chemiluminescence value from all other chemiluminescence values.
- Create a standard curve by plotting the average blank control subtracted chemiluminescence 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.
- Note: Most chemiluminescence 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 chemiluminescence 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 chemiluminescence values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at chemiluminescence 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)	RLU		Mean RLU
	1	2	
0	2,787	2,787	2,787
36.6	5,894	6,451	6,173
110	13,177	13,431	13,304
329	35,007	35,086	35,047
988	105,300	99,578	102,439
2,963	313,270	307,700	310,485
8,889	856,980	838,590	847,785
26,667	1,937,600	1,816,700	1,877,150
80,000	3,206,600	3,260,300	3,233,450

Table 1. Example of chicken Immunoglobulin Y standard curve in Sample Diluent NS. The Immunoglobulin Y 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 8.22 pg/mL. The MDD was determined by calculating the mean of zero standard replicates (n=16) and adding 2 standard deviations then extrapolating the corresponding concentration.

Recovery

Three concentrations of Immunoglobulin Y were spiked into the indicated sample diluent or biological matrix to evaluate signal recovery in the working range of the assay.

Sample Type	Average % Recovery	Range (%)
1:10,000 Mayonnaise	86	84 - 87
Antibody Diluent 4B	103	100 - 106
1X Cell Extraction Buffer PTR	98	98 - 100
1X Wash Buffer	98	87 - 104
Serum-free DMEM Media	63	52 - 77
DMEM + 10% FBS	76	70 - 87

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 Immunoglobulin Y 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: 1 Million Chicken Serum
Undiluted	ng/mL	9.42
	% Expected value	100.0
2	ng/mL	4.97
	% Expected value	105.6
4	ng/mL	2.42
	% Expected value	102.6
8	ng/mL	1.15
	% Expected value	97.5
16	ng/mL	0.60
	% Expected value	102.4
32	ng/mL	0.31
	% Expected value	104.4
64	ng/mL	0.16
	% Expected value	109.1

Native Immunoglobulin Y 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	1:10,000 Mayonnaise
Undiluted	ng/mL	1.41
	% Expected value	100
2	ng/mL	0.76
	% Expected value	108
4	ng/mL	0.40
	% Expected value	115
8	ng/mL	0.18
	% Expected value	104

Precision

Mean coefficient of variations of interpolated values of Immunoglobulin Y from three concentrations of chicken serum within the working range of the assay.

	Intra-assay	Inter-assay
N=	3	3
CV (%)	2.20	4.49

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips: <https://www.abcam.com/en-us/technical-resources/guides/elisa-guide>

Technical Support

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