Mouse Total Immunoglobulin Detection ELISA Kits

IgA, IgG, IgG1, IgG2a, IgG2b, IgG3, and IgM

Catalog # 3019, 3023, 3025, 3026, 3027, 3028, and 3024

For Research Use Only - Not Human or Therapeutic Use

PRODUCT SPECIFICATIONS

DESCRIPTION:	ELISA kits to quantify total mouse IgG/IgM/IgA immunoglobulins.
FORMAT:	96-well ELISA plate with removeable strips
ASSAY TYPE:	Sandwich ELISA
ASSAY TIME:	4.5 hours
STANDARD RANGE:	100 ng/ml to 1.6 ng/ml
NUMBER OF SAMPLES:	Up to 40 (duplicate) samples/plate
SAMPLE TYPES:	Serum & Plasma (pre-treatment acceptable)
RECOMMENDED SAMPLE DILUTIONS:	Varies (Starting point 1:100 or greater)
CHROMOGEN:	OPD (read at 490 nm)
STORAGE:	-20°C for 12 months
VALIDATION DATA:	3019 (IgA): Intra-Assay (3.2-7.9%)/Inter-Assay (3.3-8.5%)/Spiking Test (102-104%)
	3023 (IgG): Intra-Assay (4.0-10.1%)/Inter-Assay (4.2-5.7%)/Spiking Test (93-113%)
	3025 (IgG1): Intra-Assay (1.6-8.0%)/Inter-Assay (2.8-7.1%)/Spiking Test (93-100%)
	3026 (IgG2a): Intra-Assay (8.3-9.0%)/Inter-Assay (4.1-6.9%)/Spiking Test (84-102%)
	3027 (IgG2b): Intra-Assay (4.1-7.5%)/Inter-Assay (5.2-9.1%)/Spiking Test (93-100%)
	3028 (IgG3): Intra-Assay (0.9-9.7%)/Inter-Assay (0.8-3.6%)/Spiking Test (86-97%)
	3024 (IgM): Intra-Assay (2.3-6.5%)/Inter-Assay (2.7-5.1%)/Spiking Test (94-112%)
NOTES:	N/A

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INTRODUCTION

Animals are consistently exposed to environmental factors that penetrate the body from multiple sites, such as the respiratory and digestive tracts, or through damaged skin. Penetrating antigens can activate the humoral immune response which produces anti-antigen immunoglobulins to protect the host. Immunoglobulins are classified by their isotypes which differ by biological properties, functional locations, and ability to deal with different antigens. The main isotypes are summarized below:

1) Immunoglobulin A (IgA) plays a critical role in mucosal immunity and is found in the mucous membranes, especially the respiratory and gastrointestinal tracts, as well as in saliva and tears.

2) Immunoglobulin E (IgE) is involved in allergic reactions and is found in the lungs, skin, and mucous membranes.

3) Immunoglobulin G (IgG) is the most abundant isotype and is found in blood and extracellular fluids. In mice, IgG is categorized into four subtypes: IgG1, IgG2a, IgG2b, and IgG3.

4) Immunoglobulin M (IgM) is the first isotype produced in a humoral immune response and is found mainly in the blood and lymph fluid.

Total immunoglobulin isotype levels correlate with health or pathological conditions such as hypo- or hyper-gammaglobulinemia, and acute or chronic infections (1-5). In addition, total immunoglobulin levels are often analyzed relative to antigen-specific antibody levels to evaluate immune function in patients and animal disease models. Chondrex, Inc. provides mouse total immunoglobulin isotype ELISA kits for IgA, IgE, IgM, and IgG, as well as IgG subtypes: IgG1, IgG2a, IgG2b, and IgG3, along with antigen-specific antibody ELISA kits, such as autoantigens (ssDNA and dsDNA), dietary proteins (collagen and OVA), and bacteria (*E. coli*, LPS and *S. aureus*). These total immunoglobulin detection ELISA kits are also suitable for quantifying monoclonal antibody and polyclonal antibody isotypes and/or subtypes, as well as establishing reliable methods for monitoring antibody production and quality control.

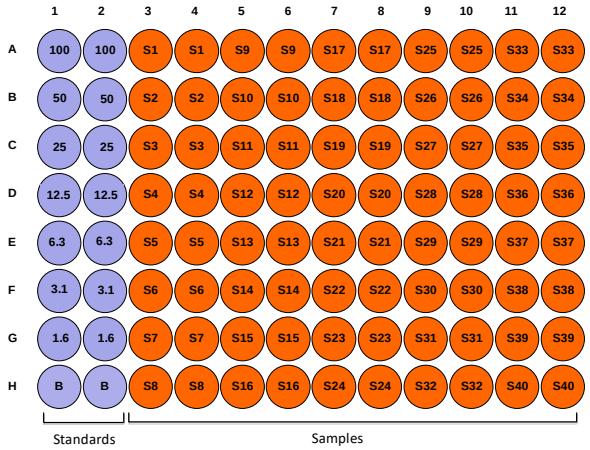
LIST OF MOUSE TOTAL IMMUNOGLOBULIN DETECTION ELISA KITS

Kit	Catalog #
Total IgE Detection ELISA Kit	3005
Total IgA Detection ELISA Kit	3019
Total IgG Detection ELISA Kit	3023
Total IgM Detection ELISA Kit	3024
Total IgG1 Detection ELISA Kit	3025
Total IgG2a Detection ELISA Kit	3026
Total IgG2b Detection ELISA Kit	3027
Total IgG3 Detection ELISA Kit	3028

KIT COMPONENTS

Item		Quantity	Amount	Storage
Standard	IgA (30181) IgG (30111) IgG1 (30131) IgG2a (30151) IgG2b (30161) IgG3 (30281) IgM (30171)	1 vial	Lyophilized 1000 ng	-20°C
Capture Antibody	IgA (30192) IgG (30232) IgG1 (30232) IgG2a (30232) IgG2b (30232) IgG3 (30282) IgM (30242)	1 vial	1 mg/ml, 100 µl	-20°C
Detection Antibody (peroxidase-conjugated polyclonal antibodies)	IgA (30193) IgG (30233) IgG1 (30133) IgG2a (30153) IgG2b (30163) IgG3 (203017) IgM (30173)	2 vials	50 µl	-20°C
Solution A - Coating Buffer (9052)		1 bottle	10 ml	-20°C
Solution B - Blocking Buffer (30235)		1 bottle	10 ml	-20°C
Solution C - Standard/Sample/Detection Antibody Dilution Buffer (30236)		1 bottle	50 ml	-20°C
OPD (90021)		2 vials	Lyophilized	-20°C
Chromogen Dilution Buffer (90022)		1 bottle	20 ml	-20°C
Stop Solution - 2N Sulfuric Acid (9016)		1 bottle	10 ml	-20°C
Wash Buffer, 20X (9005)		1 bottle	50 ml	-20°C
ELISA Plate		1 each	8-well Strips x12	-20°C

PLATE MAPPING



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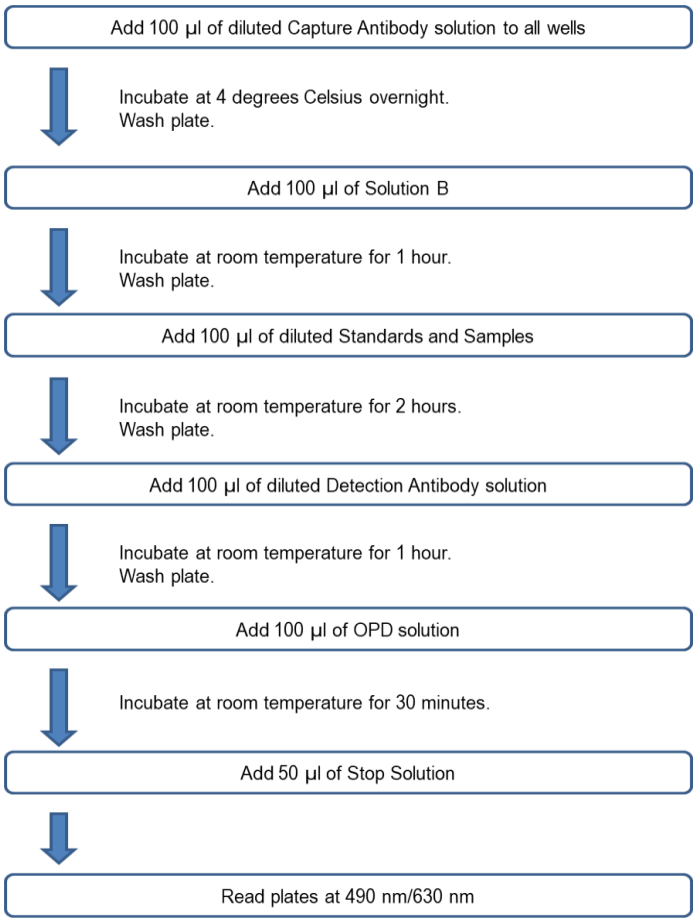
ASSAY OUTLINE



NOTES BEFORE USING ASSAY

NOTE 1: It is recommended that the standard and samples be run in duplicate.

NOTE 2: Warm up all buffers to room temperature before use.

NOTE 3: Crystals may form in Wash Buffer, 20X when stored at cold temperatures. If crystals have formed, warm the wash buffer by placing the bottle in warm water until crystals are completely dissolved.

NOTE 4: Measure exact volume of buffers using a serological pipet, as extra buffer is provided.

NOTE 5: Cover the plate with plastic wrap or a plate sealer after each step to prevent evaporation from the outside wells of the plate.

NOTE 6: For partial reagent use, please see the assay protocol's corresponding step for the appropriate dilution ratio. For example, if the protocol dilutes 50 µl of a stock solution in 10 ml of buffer for 12 strips, then for 6 strips, dilute 25 µl of the stock solution in 5 ml of buffer. Partially used stock reagents may be kept in their original vials and stored at -20ºC for use in a future assay.

NOTE 7: This kit contains animal components from non-infectious animals and should be treated as potential biohazards in use and for disposal.

ASSAY PROCEDURE

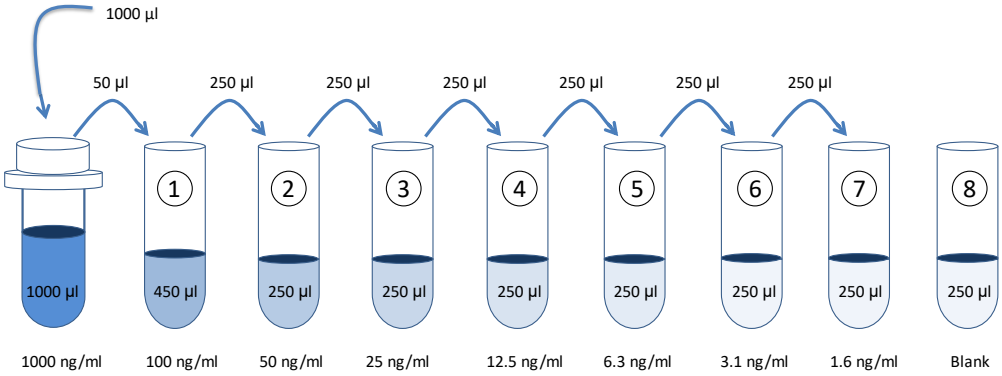
1. **Add Capture Antibody**: Centrifuge the Capture Antibody vial at 3000 rpm x 1 minute. Dilute one vial of Capture Antibody with 10 ml of Coating Buffer (Solution A). Add 100 µl of capture antibody solution to each well and incubate at 4°C overnight.

Strip #	Capture Antibody (µl)	Solution A (ml)
2	17	1.7
4	33	3.3
6	50	5.0
8	66	6.6
10	82	8.2
12	100	10.0

2. **Wash**: Dilute 50 ml of 20X wash buffer in 950 ml of distilled water (1X wash buffer). Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blotting on a paper towel to remove excess liquid. *Do not allow the plate to dry out*.

3. **Add Blocking Buffer**: Add 100 µl of the Blocking Buffer (Solution B) to each well and incubate at room temperature for 1 hour.

4. **Prepare Standard Dilutions**: The recommended standard range is 1.6-100 ng/ml. Dissolve one vial of Mouse Immunoglobulin Standard in 1 ml of Sample/Standard/Detection Antibody Dilution Buffer (Solution C) to make a 1000 ng/ml standard stock solution and then dilute the standard stock solution 1:10 with Solution C. For example, mix 50 µl of the standard stock solution (1000 ng/ml) with 450 µl of Solution C to make a 100 ng/ml solution. Then take 250 µl of the diluted standard solution and mix with 250 µl of Solution C to make a 50 ng/ml solution, and then repeat it five more times for 25, 12.5, 6.3, 3.1, and 1.6 ng/ml solutions. The remaining 1000 ng/ml standard stock solution may be stored at -20°C for use in a second assay. Chondrex, Inc. recommends making fresh serial dilutions for each assay.



5. **Prepare Sample Dilutions**: Several factors may influence immunoglobulin levels in serum, such as the adjuvant used for immunization and the timing of serum collection. In general, it is advisable to make several dilutions of your sample to ensure that the sample OD values are within the range of the standard curve.

6. **Wash**: Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blotting on a paper towel to remove excess liquid. *Do not allow the plate to dry out*.

7. **Add Standards and Samples**: Add 100 µl of standards, Solution C (blank), and samples to wells in duplicate. Incubate at room temperature for 2 hours

8. **Wash**: Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blotting on a paper towel to remove excess liquid. *Do not allow the plate to dry out*.

9. **Add Detection Antibody**: Centrifuge the Detection Antibody vial at 3000 rpm x 1 minute. Dilute one vial of Detection Antibody with 10 ml Sample/Standard/Detection Antibody Dilution Buffer (Solution C). Add 100 µl of detection antibody solution to each well and incubate at room temperature for 1 hour.

Strip #	Detection Antibody (µl)	Solution C (ml)
2	8	1.7
4	17	3.3
6	25	5.0
8	33	6.6
10	42	8.2
12	50	10.0

10. **Wash**: Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blotting on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

11. **Add OPD**: Dissolve one vial of OPD in 10 ml of Chromogen Dilution Buffer. Add 100 µl of OPD solution to each well immediately after washing the plate. Incubate for 30 minutes at room temperature.

12. **Stop**: Add 50 ml of 2N sulfuric acid (Stop Solution) to each well.

13. **Read Plate**: Read the OD values at 490 nm (A 630 nm filter can be used as a reference). If the OD values of samples are greater than the OD values of the highest standard, re-assay the samples at a higher dilution.

CALCULATING RESULTS

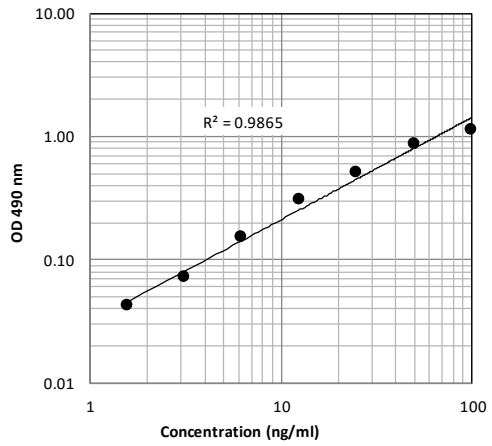
1. Average the duplicate OD values for the blank (B), standards, and test samples.

2. Subtract the averaged OD blank values from the averaged OD values of the standards and test samples.

3. Plot the OD values of standards against the concentration of standard antibody (ng/ml). Using a log/log plot will linearize the data. Figure 1 shows a representative experiment where the standard range is 1.6 - 100 ng/ml.

4. The ng/ml of antibody in test samples can be calculated using regression analysis. Multiply it by the sample dilution factor to obtain the antibody concentration (ng/ml) in the original sample specimens.

Figure 1 - A Typical Standard Curve for the Mouse Total IgG Antibody Detection ELISA Kit



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ASSAY VALIDATION

Table 1 - Reproducibility Data for the Mouse Total IgA Antibody Detection ELISA Kit (Catalog # 3019)

Test	10 ng/ml	20 ng/ml	40 ng/ml
Intra-Assay CV (%)	7.9	3.2	6.3
Inter-Assay CV (%)	6.7	8.5	3.3
Spiking Test*	102%	104%	103%

Table 2 - Reproducibility Data for the Mouse Total IgG Antibody Detection ELISA Kit (Catalog # 3023)

Test	3.1 ng/ml	12.5 ng/ml	50 ng/ml
Intra-Assay CV (%)	10.1	8.0	4.0
Inter-Assay CV (%)	4.5	4.2	5.7
Spiking Test*	93%	104%	113%

Table 3 - Reproducibility Data for the Mouse Total IgG1 Antibody Detection ELISA Kit (Catalog # 3025)

Test	3.1 ng/ml	12.5 ng/ml	50 ng/ml
Intra-Assay CV (%)	7.6	1.6	8.0
Inter-Assay CV (%)	7.1	2.8	3.6
Spike Test* (%)	93%	100%	95%

Table 4 - Reproducibility Data for the Mouse Total IgG2a Antibody Detection ELISA Kit (Catalog # 3026)

Test	3.1 ng/ml	12.5 ng/ml	50 ng/ml
Intra-Assay CV (%)	8.3	9.0	9.0
Inter-Assay CV (%)	6.3	6.9	4.1
Spike Test* (%)	102%	84%	91%

Table 5 - Reproducibility Data for the Mouse Total IgG2b Antibody Detection ELISA Kit (Catalog # 3027)

Test	3.1 ng/ml	12.5 ng/ml	50 ng/ml
Intra-Assay CV (%)	4.9	7.5	4.1
Inter-Assay CV (%)	7.6	9.1	5.2
Spike Test* (%)	100%	94%	93%

Table 6 - Reproducibility Data for the Mouse Total IgG3 Antibody Detection ELISA Kit (Catalog # 3028)

Test	3.1 ng/ml	12.5 ng/ml	50 ng/ml
Intra-Assay CV (%)	9.7	5.2	0.9
Inter-Assay CV (%)	3.6	0.8	1.5
Spike Test* (%)	97%	86%	87%

Table 7 - Reproducibility Data for the Mouse Total IgM Antibody Detection ELISA Kit (Catalog # 3024)

Test	3.1 ng/ml	12.5 ng/ml	50 ng/ml
Intra-Assay CV (%)	6.5	2.3	4.2
Inter-Assay CV (%)	5.1	4.8	2.7
Spike Test* (%)	94%	111%	112%

* Known amounts of immunoglobulins were added to samples and then diluted with Sample/Standard/Detection Antibody Dilution Buffer (Solution C).

TROUBLESHOOTING

For frequently asked questions about assays and ELISAs, please see Chondrex, Inc.'s ELISA FAQ for more information.

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