Mouse Total Immunoglobulin Detection Kit
IgG, IgG1, IgG2a, IgG2b, IgG3, and IgM

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

For Research Use Only - Not Human or Therapeutic Use

INTRODUCTION

Animals are continuously exposed to foreign antigens including environmental factors that penetrate the body from multiple sites, such as the respiratory and digestive tracts, or through damaged skin. Penetrating antigens activate the humoral immune response which produces anti-antigen immunoglobulins protecting the host. The immunoglobulins are classified by their isotypes which differ in their 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 involed 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 to 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 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 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.

Table 1 - List of mouse total immunoglobulin ELISA kits

Products	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

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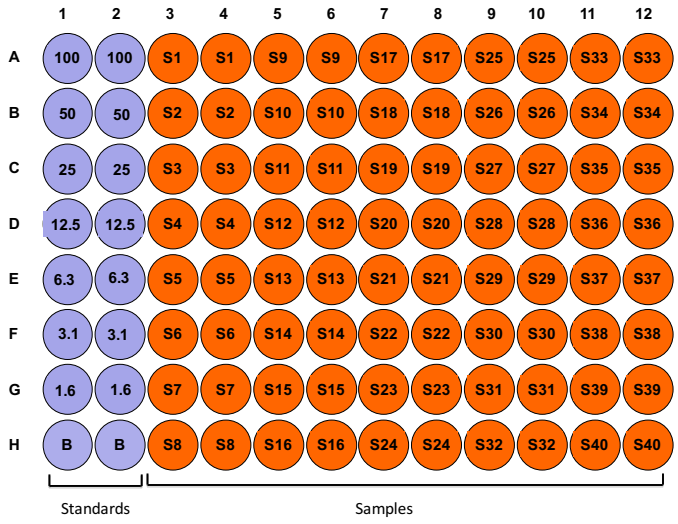
KIT COMPONENTS

Item		Quantity	Amount	Storage
Standard Mouse Immunoglobulin	IgG (30111) IgG1 (30131) IgG2a (30151) IgG2b (30161) IgG3 (30281) IgM (30171)	1 vial	1000 ng/vial, lyophilized	-20°C
Capture Antibody	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	IgG (30233) IgG1 (30133) IgG2a (30153) IgG2b (30163) IgG3 (203017) IgM (30173)	2 vials	50 µl	-20°C
Solution A- Capture Antibody Dilution Buffer (9052)		1 bottle	10 ml	-20°C
Solution B - Blocking Buffer (30235)		1 bottle	10 ml	-20°C
Solution C - Sample/Standard/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 (9016)		1 bottle	10 ml	-20°C
Wash Buffer, 20X (9005)		1 bottle	50 ml	-20°C
ELISA Plate		1 each	96-well (8-well strips x 12)	-20°C

Figure 1 - A Standard Assay Layout



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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: Partially used reagents may be kept at –20°C.

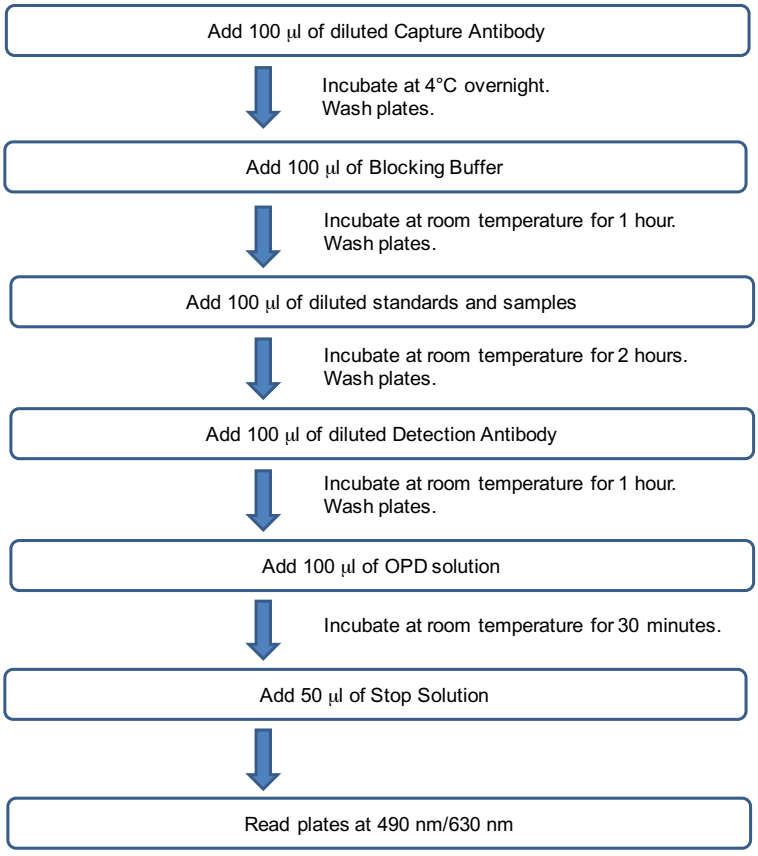
Note 4: Crystals may form in the 20X wash buffer when stored at cold temperatures. If crystals have formed, warm the wash buffer by placing the bottle in warm water until crystals are dissolved completely.

Note 5: Measure exact volume of buffers using a serological pipette, as extra buffer is provided.

Note 6: Cover the plate with plastic wrap or a plate sealer after each step to prevent evaporation from the outside wells of the plate.

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

ASSAY OUTLINE



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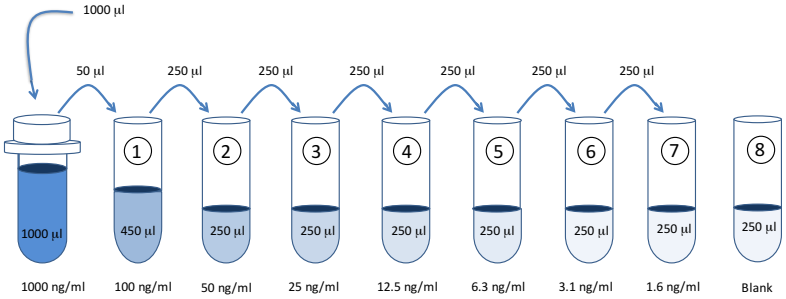
1. **Add Capture Antibody**: Centrifuge the Capture Antibody vial at 3000 rpm x 1 minute. Dilute one vial of Capture Antibody with 10 ml of Capture Antibody Dilution Buffer (Solution A). Add 100 µl of capture antibody solution to each well and incubate at 4°C overnight.

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 blot 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 solution and keep it at -20°C for future assays. Next, dilute the standard 1:10 with Solution C. For example, mix 50 µl of the standard (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, 6.25, 3.125, and 1.6 ng/ml solutions. The remaining 1000 ng/ml standard stock may be stored at -20°C for use in a second assay. We recommend 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 standard curve range.

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 blot 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 blot 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.

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 blot 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 µl 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.

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CALCULATION OF ANTIBODY TITERS

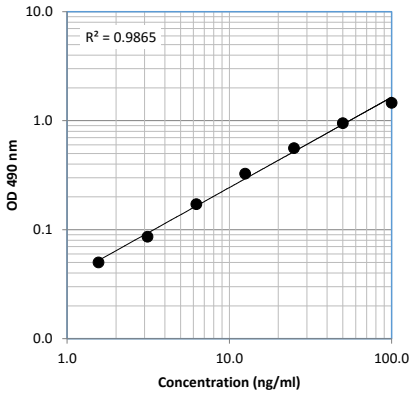
1. Average the duplicate OD values for the standards, blanks (B), and test samples.

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

3. Plot the OD values of the standards against the concentration of antibody standard (ng/ml). Using a log/log plot will linearize the data. Figure 2 show an example of a standard curve of total IgG ELISA.

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 original sample specimens.

Figure 2 - A typical standard curve for mouse total IgG ELISA



PRECISION TEST RESULTS

Table 2 - Precision of Mouse Total IgG ELISA Kit (Catalog # 3023)

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

Table 3 - Precision of Mouse Total IgG1 ELISA Kit (Catalog # 3025)

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

Table 4 - Precision of Mouse Total IgG2a ELISA Kit (Catalog # 3026)

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

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Table 5 - Precision of Mouse Total IgG2b ELISA Kit (Catalog # 3027)

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

Table 6 - Precision of Mouse Total IgG3 ELISA Kit (Catalog # 3028)

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

Table 7 - Precision of Mouse Total IgM ELISA Kit (Catalog # 3024)

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

*Standard was added with known amounts of immunoglobulin and then diluted with Sample/Standard/Detection Antibody Dilution Buffer to assay total immunoglobulin by ELISA.

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