Mouse Anti-*Acarus siro* Antibody Subtype/Subclass ELISA Kits

Catalog # 3097, 3098, 3099, and 3100

For Research Use Only - Not Human or Therapeutic Use

PRODUCT SPECIFICATIONS

DESCRIPTION:	ELISA kits to quantify mouse anti-*Acarus siro* antibodies
FORMAT:	Precoated 96-well ELISA Plate with removeable strips
ASSAY TYPE:	Indirect ELISA
ASSAY TIME:	4.5 hours
STANDARD RANGE:	3097 (IgG) : 10 - 0.16 ng/ml
	3098 (IgG1) : 10 - 0.16 ng/ml
	3099 (IgG2a) : 5 - 0.08 ng/ml
	3100 (IgM) : 1000 - 16 ng/ml
NUMBER OF SAMPLES:	Up to 40 (duplicate) samples/plate
SAMPLE TYPES:	Serum & Plasma (pre-treatment acceptable)
RECOMMENDED SAMPLE DILUTIONS:	1:100 (at least)
CHROMOGEN:	TMB (read at 450 nm)
STORAGE:	-20°C for 12 months
VALIDATION DATA:	3097 (IgG): Intra-Assay (2.7-6.5%)/Inter-Assay (1.6-8.1%)/Spiking Test (96-100%)
	3098 (IgG1): Intra-Assay (3.7-9.0%)/Inter-Assay (2.7-8.9%)/Spiking Test (93-110%)
	3099 (IgG2a): Intra-Assay (3.0-5.8%)/Inter-Assay (5.9-9.2%)/Spiking Test (96-101%)
	3100 (IgM): Intra-Assay (5.0-8.6%)/Inter-Assay (1.4-7.6%)/Spiking Test (93-98%)
NOTES:	N/A

16928 Woodinville-Redmond Rd NE Suite B-101
Woodinville, WA 98072
Phone: 425.702.6365 or 888.246.6373
Fax: 425.882.3094
www.chondrex.com
support@chondrex.com

Mouse Anti-*Acarus siro* Antibody Subtype/Subclass ELISA Kits

Catalog # 3097, 3098, 3099, and 3100

For Research Use Only - Not Human or Therapeutic Use

INTRODUCTION

Storage dust mites (SDM) are commonly found in stored foods such as grain, flour, and hay. They are known to cause symptoms such as bronchial asthma, allergic rhinitis, and conjunctivitis, particularly in rural work environments. SDM thrive in warm, humid conditions with an optimum relative humidity of 80% and temperatures between 25 and 30°C.

Asthma and other respiratory diseases caused by sensitization to dust mites are global health problems. Because mites are found in both temperate and subtropical climates, a large proportion of the world's population is exposed. In rural areas of Europe, species such as *Lepidoglyphus destructor* and *Acarus siro* have been identified as significant contributors to these allergies.

In human patients, researchers have identified five IgE-binding components in *Acarus siro* extracts with molecular weights ranging from 15 to 22 kDa. Among the allergens from *Acarus siro* group 13 allergens are fatty acid-binding proteins with a molecular weight of 15 kDa. These proteins bind and transport fatty acids (1) and are recognized by 23% of patients with *Acarus siro*-related allergies (2).

In animal studies, exposure of rats to *Acarus siro* feces significantly increased serum levels of IFN-gamma and IgE compared to controls. The treated rats also had lower serum IL-4 levels and a higher percentage of eosinophils and basophils in the leukocyte differential count (3).

To study the immune response to allergens and allergen-specific pathological effects in mouse models, Chondrex, Inc. provides mouse anti-*Acarus siro* antibody ELISA kits. Chondrex, Inc. also offers ELISA kits for assaying other mite and mite-allergen antibody subtypes/subclasses and total immunoglobulin subtypes/subclasses. Please visit www.chondrex.com for more information.

LIST OF MOUSE ANTI-MITE ANTIBODY SUBTYPE/SUBCLASS ELISA KITS

Kit	*Acarus siro* Catalog #	HDM Der p Catalog #	Der p1 Catalog #	Der p2 Catalog #	HDM Der f Catalog #
Mouse IgG Antibody ELISA Kit	3097	3030	3047	3065	3072
Mouse IgG1 Antibody ELISA Kit	3098	3034	3048	Coming soon!	3073
Mouse IgG2b Antibody ELISA Kit	Coming soon!	3035	Coming soon!	3067	3074
Mouse IgM Antibody ELISA Kit	3100	3036	3049	3068	3076
Mouse IgE Antibody ELISA Kit	Coming soon!	3037	Coming soon!	Coming soon!	3081
Mouse IgG2a Antibody ELISA Kit	3099	3038	Coming soon!	3066	Coming soon!
Mouse IgG3 Antibody ELISA Kit	Coming soon!	3039	3064	Coming soon!	3075
Mouse IgA Antibody ELISA Kit	Coming soon!	3046	Coming soon!	Coming soon!	Coming soon!

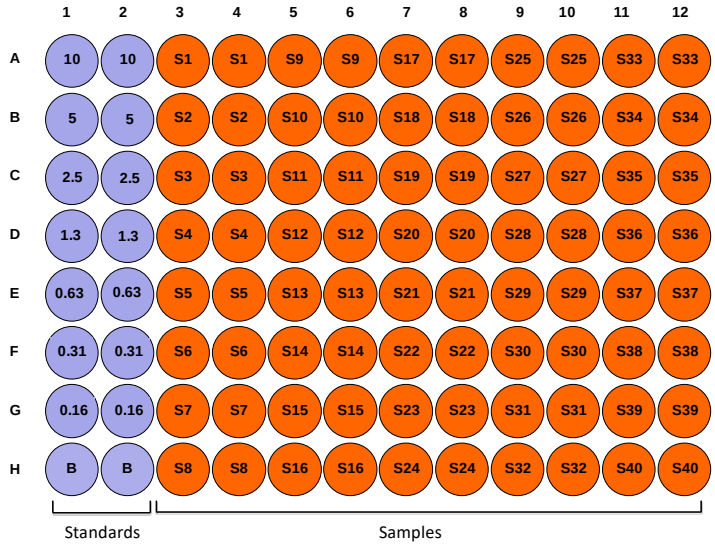
16928 Woodinville-Redmond Rd NE Suite B-101
Woodinville, WA 98072
Phone: 425.702.6365 or 888.246.6373
Fax: 425.882.3094
www.chondrex.com
support@chondrex.com

KIT COMPONENTS

Item		Quantity	Amount	Storage
Standard	IgG (30971) - 10 ng IgG1 (30981) - 10 ng IgG2a (30991) - 5 ng IgM (31001) - 1000 ng	1 vial	Lyophilized	-20°C
Secondary Antibody (peroxidase-conjugated polyclonal antibodies)	IgG (7012) IgG1 (62072) IgG2a (62102) IgM (30173)	2 vials	50 µl	-20°C
Solution B – Blocking/Sample/Standard Dilution Buffer (67015)		1 bottle	50 ml	-20°C
Solution C – Secondary Antibody Dilution Buffer (2073)		1 bottle	10 ml	-20°C
TMB Solution (90023)		2 vials	0.2 ml	-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
Acarus siro Extract coated ELISA Plate (Blue)		1 each	96-well (8-well strips x 12)	-20°C

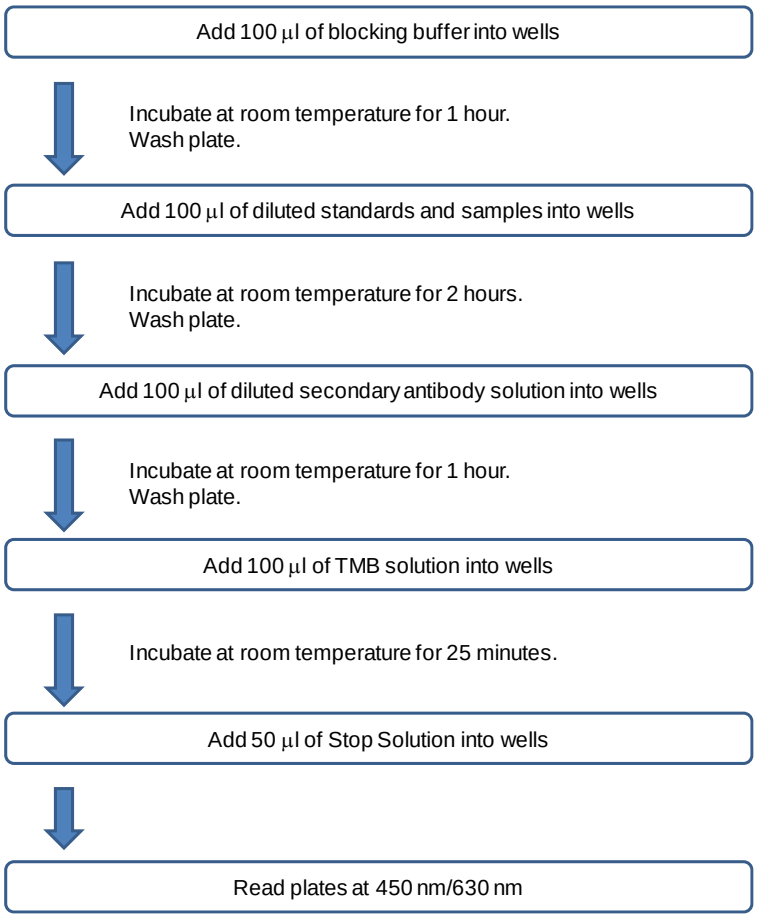
PLATE MAPPING

Example of the Mouse Anti-*Acarus siro* IgG Antibody ELISA Kit



16928 Woodinville-Redmond Rd NE Suite B-101
Woodinville, WA 98072
Phone: 425.702.6365 or 888.246.6373
Fax: 425.882.3094
www.chondrex.com
support@chondrex.com

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.

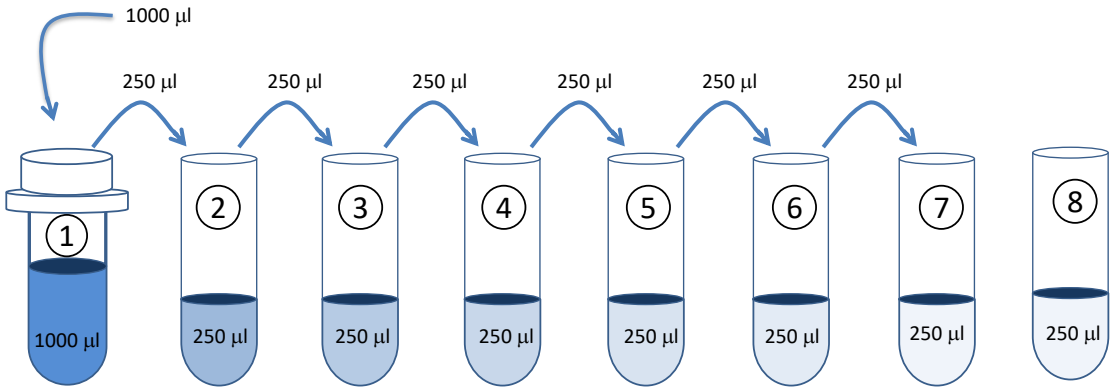
NOTE 6: For partial reagent use, please see the assay protocol's corresponding step for the appropriate dilution ratio. For example, if the protocol dilutes 50 µl of a stock solution in 10 ml of buffer for 12 strips, then for 6 strips, dilute 25 µl of the stock solution in 5 ml of buffer. Partially used 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 Blocking Buffer**: Add 100 µl of the Blocking/Sample/Standard Dilution Buffer (Solution B) to each well and incubate at room temperature for 1 hour.

2. **Prepare Standard Dilutions**: Please see the figure below for each assay's recommended standard range. Dissolve one vial of Standard in 1 ml of Blocking/Sample/Standard Dilution Buffer (Solution B) and keep it as a standard stock. Then serially dilute it with Solution B. For example, mix 250 µl of the stock solution with an equal volume of Solution B to make the second stock solution, and then repeat it five more times. The remaining stock solution can be stored at -20ºC for use in a future assay. Chondrex, Inc. recommends making fresh serial dilutions for each assay.



IgG/IgG1:	10 ng/ml	5 ng/ml	2.5 ng/ml	1.25 ng/ml	0.63 ng/ml	0.31 ng/ml	0.16 ng/ml	Blank
IgG2a:	5 ng/ml	2.5 ng/ml	1.25 ng/ml	0.63 ng/ml	0.31 ng/ml	0.16 ng/ml	0.08 ng/ml	Blank
IgM:	1000 ng/ml	500 ng/ml	250 ng/ml	125 ng/ml	63 ng/ml	31 ng/ml	16 ng/ml	Blank

3. **Prepare Sample Dilutions**: An important point to note is that the composition of *Acarus siro* extracts can exhibit variations depending on the vendor, batch, and manufacturing process. These variations can result in differing levels of antigens in the final *Acarus siro* extract product, which when used to immunize mice, can impact the serum antibody levels against those antigens. The dilution of mouse serum (1:100 or more) immunized with various *Acarus siro* products will vary depending on the immunization schedule and timing of serum collection. In general, no antibodies against *Acarus siro* are observed in normal serum at a 1:100 dilution. If serum samples require a lower dilution than 1:100, please contact support@chondrex.com.

4. **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.*

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

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 Secondary Antibody**: Dilute one vial of Secondary Antibody in 10 ml Secondary Antibody Dilution Buffer (Solution C). Add 100 µl of secondary antibody solution to each well and incubate at room temperature for 1 hour.

Strip #	2nd 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

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 TMB Solution**: Use new tubes when preparing TMB solution. Just prior to use, prepare TMB solution with Chromogen Dilution Buffer as shown in the following table. Add 100 µl of TMB solution to each well immediately after washing the plate and incubate for 25 minutes at room temperature.

Strip #	TMB (µl)	Chromogen Dilution Buffer (ml)
2	34	1.7
4	66	3.3
6	100	5.0
8	132	6.6
10	164	8.2
12	200	10.0

10. **Stop**: Stop the reaction with 50 µl of 2N Sulfuric Acid (Stop Solution) to each well.

11. **Read Plate**: Read the OD values at 450 nm. If the OD values of samples are greater than the OD values of the highest standard, re-assay the samples at a higher dilution. A 630 nm filter can be used as a reference.

CALCULATING RESULTS

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

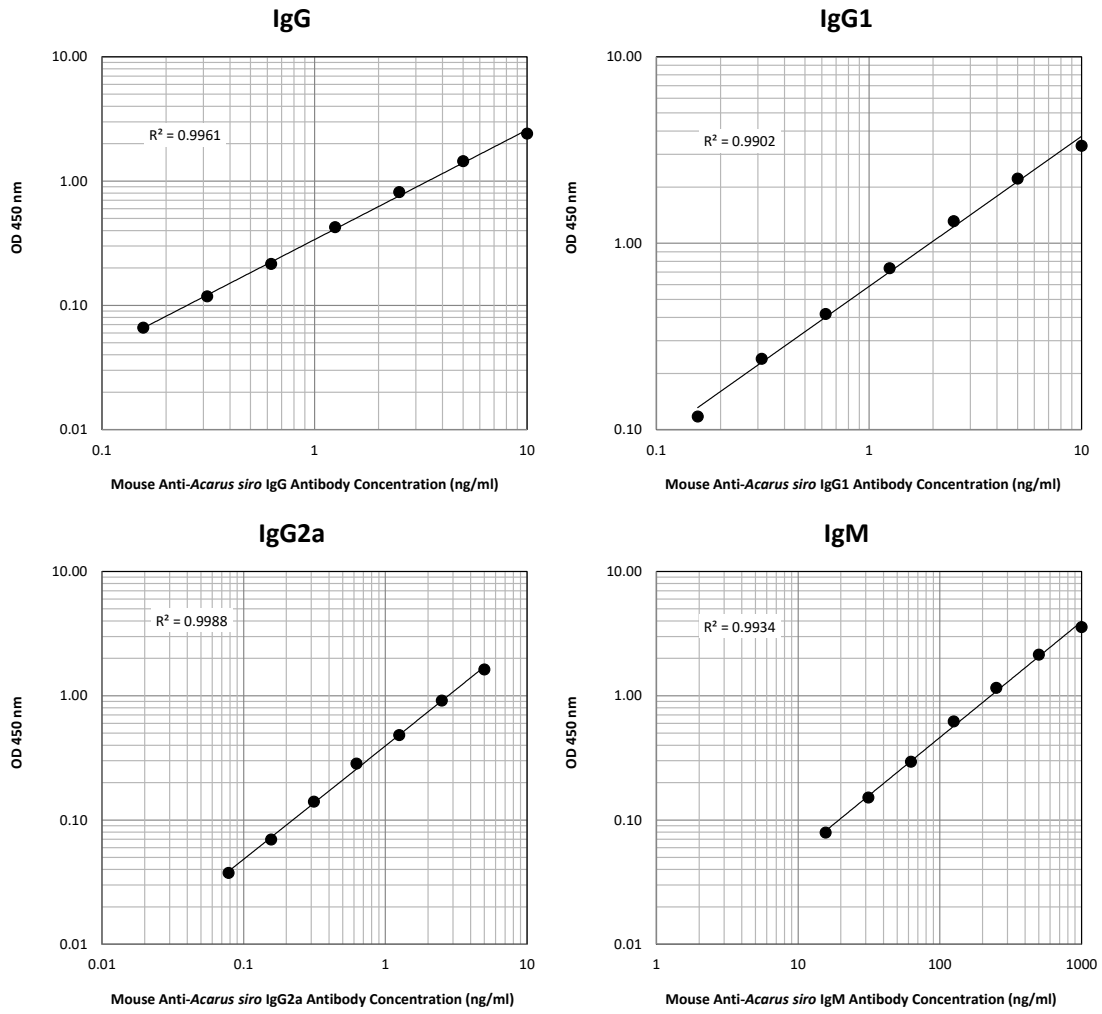
2. Subtract the averaged blank OD values from the averaged OD values of the standards and test samples.

3. Plot the OD values of the standards against the ng/ml of antibody standard. Using a log/log plot will linearize the data. Figure 1 shows examples of standard curves for anti-*Acarus siro* antibodies.

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 in original test samples.

16928 Woodinville-Redmond Rd NE Suite B-101
Woodinville, WA 98072
Phone: 425.702.6365 or 888.246.6373
Fax: 425.882.3094
www.chondrex.com
support@chondrex.com

Figure 1 - Typical Standard Curves for the Anti-*Acarus siro* Antibody ELISA Kits



VALIDATION DATA

Table 1 - Reproducibility Data for the Mouse Anti-*Acarus siro* IgG Antibody ELISA Kit

Test	0.32 ng/ml	1.25 ng/ml	5 ng/ml
Intra-Assay CV (%)	2.7	6.5	3.6
Inter-Assay CV (%)	1.6	1.7	8.1
Spike Test* (%)	99%	96%	100%

Table 2 - Reproducibility Data for the Mouse Anti-*Acarus siro* IgG1 Antibody ELISA Kit

Test	0.32 ng/ml	1.25 ng/ml	5 ng/ml
Intra-Assay CV (%)	9.0	3.7	4.0
Inter-Assay CV (%)	2.7	8.9	5.7
Spike Test* (%)	100%	93%	110%

Table 3 - Reproducibility Data for the Mouse Anti-*Acarus siro* IgG2a Antibody ELISA Kit

Test	0.16 ng/ml	0.63 ng/ml	2.5 ng/ml
Intra-Assay CV (%)	5.8	3.0	3.6
Inter-Assay CV (%)	7.0	5.9	9.2
Spike Test* (%)	99%	96%	101%

Table 4 - Reproducibility Data for the Mouse Anti-*Acarus siro* IgM Antibody ELISA Kit

Test	32 ng/ml	125 ng/ml	500 ng/ml
Intra-Assay CV (%)	7.5	8.6	5.0
Inter-Assay CV (%)	7.6	1.6	1.4
Spike Test* (%)	93%	98%	95%

*Known amounts of anti-*Acarus siro* antibodies were added to samples and then diluted with Blocking/Sample/Standard Dilution Buffer to assay anti-*Acarus siro* antibodies by ELISA.

TROUBLESHOOTING

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

REFERENCES

1. T. Eriksson, P. Whitley, E. Johansson, H. van, G. Gafvelin, Identification and characterisation of two allergens from the dust mite Acarus siro, homologous with fatty acid-binding proteins. *Int Arch Allergy Immunol* **119**(4), 275-81 (1999).

2. C. Luczynska, P. Griffin, R. Davies, M. Topping, Prevalence of specific IgE to storage mites (A. siro, L. destructor and T. longior) in an urban population and crossreactivity with the house dust mite (D. pteronyssinus). *Clin Exp Allergy* **20**(4), 403-6 (1990).

3. B. Abdel-Salam, Seasonal population of Acarus siro mites and effects of their faeces on allergenic immunological disorder modulated by garlic in albino rat. *Allergol Immunopathol (Madr)* **40**(3), 144-51 (2012).

16928 Woodinville-Redmond Rd NE Suite B-101
Woodinville, WA 98072
Phone: 425.702.6365 or 888.246.6373
Fax: 425.882.3094
www.chondrex.com
support@chondrex.com