

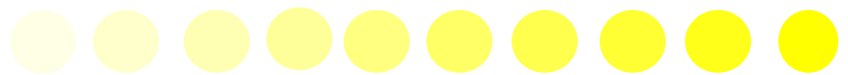
ELISA 2.0

Quantitative Allergen Immunoassay Kit

- White Paper -

June 2020

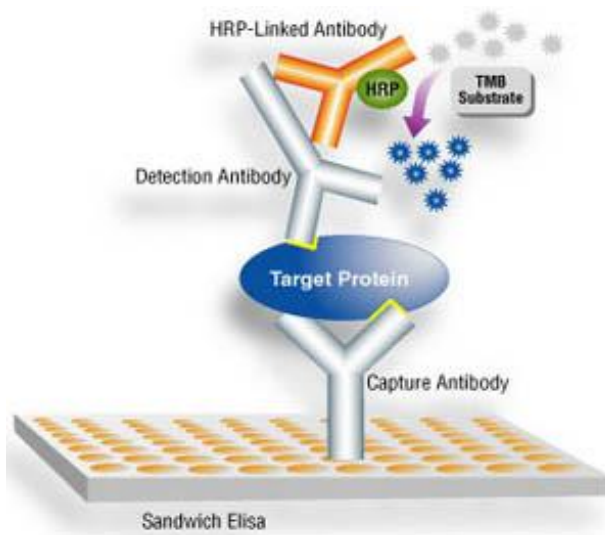
Developed and Manufactured by



INTRODUCTION

Enzyme Linked Immunosorbent Assay (ELISA) was developed in the early 1970's as an antibody-based method for measuring specific analytes in biological and biomedical systems. Because of the relative ease of use, potential for high throughput screening, and readily available reagents, ELISA has become a standard technique used worldwide with applications ranging from antibody and cytokine testing to environmental allergen and food safety testing.

While ELISA formats vary in complexity (indirect, sandwich, competitive), they all share common characteristics:



- 1) antibody or antigen immobilized onto a solid surface (e.g. a 96-well microtiter plate) to capture a specific analyte in solution
- 2) enzyme-conjugated antibody with specificity for the captured analyte or the surface-bound antigen
- 3) enzyme substrate that generates a measurable signal by color development, luminescence, or fluorescence

Over the past 25 years, Indoor Biotechnologies has built a comprehensive portfolio of proprietary, allergen-specific antibodies to allow the development of more than 30 different ELISA kits for the detection of dust mite, cockroach, mold, pollen, animal, and food allergens. Indoor Biotechnologies' ELISA-10 kits have been used in over 35 different countries by a variety of customers, including academic and government institutions, allergen extract manufacturers, pharmaceutical and biotechnology companies, cleaning product manufacturers, and environmental testing laboratories. In recent years, the limitations of ELISA-10 kits have become apparent. The kits require the end user to provide additional chemicals and reagents that increase the likelihood of operator error and make standardization between laboratories harder to achieve.

Indoor Biotechnologies will discontinue its traditional ELISA-10 kits as of December 31, 2020 in favor of the new ELISA 2.0 kits which contain pre-coated ELISA plates together with all reagents required for the assay. ELISA 2.0 kits have superior performance and have been fully validated for analytical purposes.

ELISA 2.0 Indoor Biotechnologies' ELISA 2.0 kits include all reagents and buffers needed to perform the assay and allow the analyses to be completed in approximately 2.0 hours.

The kits are available in single and five-plate formats (25-plate format available as special order) and include the following:

- 96-well microtiter plate(s) pre-coated with capture antibody
- Calibration standard formulated from purified natural or recombinant allergen*
- Detection antibody
- Enzyme conjugate or conjugated secondary antibody (assay-dependent)
- TMB enzyme substrate
- Stop solution
- Assay buffer concentrate
- Wash buffer concentrate



*Purified allergens are manufactured by Indoor Biotechnologies using ISO 9001:2015 quality systems and are validated by mass spectrometry, monoclonal antibody and human IgE antibody binding.

ELISA 2.0 has been optimized to achieve maximum sensitivity and signal-to-noise ratio, while offering several convenience and performance advantages compared to traditional ELISA.

Get ELISA results in just 2.0 hours

Pre-coated plates eliminate the overnight incubation step and the need to plan experiments a day or more in advance. It is now possible to process, extract, and analyze samples on the same day – a great feature for time-sensitive projects or when quick turn-around-time is required. No overnight plate coating or blocking is required, and by combining the detection antibody and enzyme conjugate incubation steps, an additional hour of incubation is saved compared to traditional ELISA. This also reduces the potential for operator error.

ELISA 2.0 has been validated using Indoor Biotechnologies' purified allergens. In addition to the far shorter assay time, ELISA 2.0 assays offer enhanced sensitivity compared to traditional ELISA. Representative ELISA 2.0 performance characteristics are presented in Tables 1 through 6 (individual ELISA 2.0 assay validation reports are available upon request). A complete list of ELISA 2.0 assays is shown in the appendix on page 8.

Note: this product line is continually expanding; please visit our website at www.inbio.com/elisa-2.0 for the current ELISA 2.0 catalog.

Table 1. House Dust Mite and Storage Mite ELISA 2.0 Performance Characteristics

Allergen:	Blo t 5	Der f 1	Der f 2	Der p 1	Der p 2	Der p 23	Tyr p 2
Linearity (R²)¹	0.999	0.999	0.999	1	0.999	0.998	0.999
Range (ng/ml)²	7.5-0.12	25-0.39	62.5-0.98	100-0.78	12.5-0.1	25-0.39	250-7.81
Limit of Quantification³							
<i>LLOQ (ng/ml)^{3a}</i>	0.23-0.12	0.39-0.19	1.95-0.49	1.56-0.39	0.4-0.1	1.56-0.39	7.8-1.95
<i>ULOQ (ng/ml)^{3b}</i>	30-7.5	25-12.5	250-62.5	50-25	25-6.25	25-12.5	500-250
Accuracy (% Recovery)⁴							
<i>Intra-assay (n=9)^{4a}</i>	84-130%	88-118%	73-118%	98-117%	83-132%	87-129%	93-116%
<i>Inter-assay (n=54)^{4b}</i>	105%	106%	88%	103%	104%	101%	108%
Precision (%CV)⁵							
<i>Intra-assay (n=9)^{5a}</i>	1-16%	5-15%	1-12%	4-13%	4-14%	3-11%	3-9%
<i>Inter-assay (n=54)^{5b}</i>	8%	10%	7%	8%	7%	8%	6%

Table 2. Animal and Cockroach ELISA 2.0 Performance Characteristics

Allergen:	Can f 1	Fel d 1	Fel d 4	Mus m 1	Rat n 1	Bla g 1	Bla g 2	Bla g 5	Per a 7
Linearity (R²)¹	1	0.999	1	1	1	1	0.999	0.999	0.999
Range (ng/ml)²	25-0.39	25-0.19	5-0.04	25-0.1	12.5-0.19	50-0.39	100-0.39	125-1.95	12.5-0.19
Limit of Quantification³									
<i>LLOQ (ng/ml)^{3a}</i>	0.39	0.39-0.19	0.31-0.02	0.19-0.1	0.39-0.19	0.78-0.19	1.56-0.39	7.81-1.95	0.39-0.19
<i>ULOQ (ng/ml)^{3b}</i>	25	25-12.5	5	25-12.5	50-25	50-25	50-25	250-125	50-6.25
Accuracy (% Recovery)⁴									
<i>Intra-assay (n=9)^{4a}</i>	83-103%	96-129%	93-106%	91-113%	85-129%	71-108%	92-113%	77-113%	71-110%
<i>Inter-assay (n=54)^{4b}</i>	91%	113%	97%	103%	102%	94%	101%	102%	99%
Precision (%CV)⁵									
<i>Intra-assay (n=9)^{5a}</i>	5-18%	4-13%	4-8%	5-9%	6-12%	3-9%	5-15%	4-12%	3-13%
<i>Inter-assay (n=54)^{5b}</i>	9%	9%	6%	8%	10%	6%	10%	8%	7%

Table 3. Mold and Pollen ELISA 2.0 Performance Characteristics

Allergen:	Alt a 1	Asp f 1	Amb a 1	Bet v 1	Cry j 1	Lol p 1	Phl p 5
Linearity (R²)¹	1	1	1	0.999	1	0.994	1
Range (ng/ml)²	25-0.1	40-0.31	100-0.78	50-0.39	50-0.78	125-7.81	250-0.98
Limit of Quantification³							
<i>LLOQ (ng/ml)^{3a}</i>	0.4-0.1	0.63-0.16	1.56-0.78	0.39	0.78-0.39	15.63-3.91	1.95-0.98
<i>ULOQ (ng/ml)^{3b}</i>	25-6.25	40-20	200-25	100-50	50-25	250-125	250-62.5
Accuracy (% Recovery)⁴							
<i>Intra-assay (n=9)^{4a}</i>	90-115%	78-115%	90-108%	82-120%	94-106%	81-112%	85-120%
<i>Inter-assay (n=54)^{4b}</i>	99%	95%	100%	100%	100%	96%	103%
Precision (%CV)⁵							
<i>Intra-assay (n=9)^{5a}</i>	3-11%	4-22%	6-12%	1-9%	6-16%	7-16%	7-10%
<i>Inter-assay (n=54)^{5b}</i>	6%	12%	8%	7%	10%	12%	9%

Table 4. Peanut and Tree Nut ELISA 2.0 Performance Characteristics

Allergen:	Ara h 1	Ara h 2	Ara h 3	Ara h 6	Ara h 8	Ana o 3	Cor a 9
Linearity (R²)¹	1	0.999	0.999	1	1	1	0.998
Range (ng/ml)²	1,000-31.25	125-0.98	62.5-0.49	25-0.05	12.5-0.19	20-0.16	50-0.39
Limit of Quantification³							
<i>LLOQ (ng/ml)^{3a}</i>	31.25-15.63	3.91-0.49	1.95-0.49	0.2-0.05	1.56-0.2	0.16	0.78-0.39
<i>ULOQ (ng/ml)^{3b}</i>	1,000	250-31.25	62.5-31.25	25-12.5	25-6.25	20-5	100-12.5
Accuracy (% Recovery)⁴							
<i>Intra-assay (n=9)^{4a}</i>	82-124%	80-116%	74-127%	105-113%	100-109%	85-110%	75-112%
<i>Inter-assay (n=54)^{4b}</i>	109%	101%	97%	108%	102%	99%	93%
Precision (%CV)⁵							
<i>Intra-assay (n=9)^{5a}</i>	3-12%	7-14%	3-11%	7-13%	3-15%	4-10%	5-15%
<i>Inter-assay (n=54)^{5b}</i>	8%	10%	5%	8%	8%	8%	10%

Table 5. Food (non-nut) ELISA 2.0 Performance Characteristics

Allergen:	Shrimp							
	Api g 1	Bos d 5	Bos d 11	Gal d 1	Gal d 2	Gly m 5	Tropomyosin	Sin a 1
Linearity (R²)¹	0.999	1	1	1	1	1	0.999	0.999
Range (ng/ml)²	100-1.56	12.5-0.1	500-7.81	250-7.81	50-0.39	250-1.95	25-0.2	15-0.06
Limit of Quantification³								
<i>LLOQ (ng/ml)^{3a}</i>	6.25-1.56	0.78-0.1	7.81-1.95	7.8-3.9	0.78-0.39	3.91-1.95	0.39-0.1	0.06-0.03
<i>ULOQ (ng/ml)^{3b}</i>	200-50	25-12.5	500-125	250	100-25	250-62.5	25-6.25	15-7.5
Accuracy (% Recovery)⁴								
<i>Intra-assay (n=9)^{4a}</i>	81-131%	84-120%	80-128%	89-101%	91-132%	93-119%	95-115%	70-132%
<i>Inter-assay (n=54)^{4b}</i>	108%	103%	94%	95%	104%	105%	108%	96%
Precision (%CV)⁵								
<i>Intra-assay (n=9)^{5a}</i>	3-11%	4-9%	2-16%	8-13%	5-16%	5-11%	4-14%	3-16%
<i>Inter-assay (n=54)^{5b}</i>	7%	6%	7%	9%	11%	8%	7%	8%

1. Linearity is the mean R² of six ELISA plates for control curves generated using 4-parameter logistic fit.
2. Range is the average usable range of control curves from six ELISA plates that have a value of 70-130% of the expected concentration, with %CV < 15 between duplicate points.
3. Limit of Quantification - two distinctions
 - 3a. LLOQ - The lowest concentration points of six control curves with a recovery of 70-130% and %CV < 15, expressed as a range.
 - 3b. ULOQ - The highest concentration points of six control curves with a recovery of 70-130% and %CV < 15, expressed as a range.
4. Accuracy - two distinctions
 - 4a. Intra-assay - The range of average percent recovery of samples A, B, and C run in triplicate from six ELISA plates (n=9).
 - 4b. Inter-assay - The overall average percent recovery of samples A, B, and C run in triplicate from six ELISA plates (n=54).
5. Precision - two distinctions
 - 5a. Intra-assay - The range of average percent coefficient of variation of samples A, B, and C run in triplicate from six ELISA plates (n=9).
 - 5b. Inter-assay - The overall average percent coefficient of variation of samples A, B, and C run in triplicate from six ELISA plates (n=54).

Table 6. ELISA 2.0 Sensitivity compared to traditional ELISA

Allergen:	Der p 1	Der f 1	Fel d 1	Can f 1	Mus m 1	Rat n 1	Bla g 2	Phl p 5	Bet v 1
ELISA 2.0	0.39	0.20	0.20	0.39	0.10	0.20	0.39	0.98	0.39
Traditional ELISA	0.98	0.98	0.39	0.98	0.39	0.39	0.98	0.98	1.9

LLOQ values (ng/ml)

SUMMARY

ELISA 2.0 kits enable rapid, reliable and quantitative allergen measurements. Complete assay kits with pre-coated plates offer convenience, while validated reagents ensure consistent results and enhanced performance characteristics.

Please visit www.inbio.com to see Indoor Biotechnologies' full range of products and services.

ELISA 2.0 Complete Kits for Quantitative Allergen Detection

Convenience: **Just Add Water!***

- ✓ Kits include **all** reagents
- ✓ No buffer preparation required

Quicker Results with Pre-coated Plates

- ✓ No overnight incubation
- ✓ Be spontaneous – same day testing!
- ✓ Results in 2 hours

Improved Performance:

- ✓ Increased assay sensitivity
- ✓ Reduced potential for operator error
- ✓ Consistent performance with validated assay reagents
- ✓ Ready for regulatory compliance



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*Type I ultrapure water or 18.2MΩ de-ionized water required to dilute buffer concentrates

Appendix. Complete list of ELISA 2.0 assays (June 2020)

PRODUCT DESCRIPTION	ANTIBODY PAIRS	PRODUCT CODE	
		1 PLATE	5 PLATES
HOUSE DUST MITE ALLERGENS			
Blo t 5	Rabbit anti Blo t 5/Rabbit anti Blo t 5	EPC-BT5-1	EPC-BT5-5
Der f 1	6A8/4C1	EPC-DF1-1	EPC-DF1-5
Der f 2	1D8/7A1	EPC-DF2-1	EPC-DF2-5
Der p 1	5H8/4C1	EPC-DP1-1	EPC-DP1-5
Der p 2	1D8/7A1	EPC-DP2-1	EPC-DP2-5
Der p 23	Rabbit anti Der p 23/7A8	EPC-DP23-1	EPC-DP23-5
STORAGE MITE ALLERGENS			
Tyr p 2	Rabbit anti Tyr p 2/Rabbit anti Tyr p 2	EPC-BG1-1	EPC-BG1-5
COCKROACH ALLERGENS			
Bla g 1	10A6/Rabbit anti Bla g 1	EPC-BG1-1	EPC-BG1-5
Bla g 2	7C11/Rabbit anti Bla g 2	EPC-BG2-1	EPC-BG2-5
Bla g 5	17B12/Rabbit anti Bla g 5	EPC-BG5-1	EPC-BG5-5
Per a 7	Rabbit anti Shrimp Tropomyosin/1A6	EPC-PA7-1	EPC-PA7-5
ANIMAL ALLERGENS			
Can f 1	10D4/Rabbit anti Can f 1	EPC-CF1-1	EPC-CF1-5
Fel d 1	6F9/3E4	EPC-FD1-1	EPC-FD1-5
Fel d 4	5F3/Rabbit anti Fel d 4	EPC-FD4-1	EPC-FD4-5
Mus m 1	Rabbit anti Mus m 1/Rabbit anti Mus m 1	EPC-MM1-1	EPC-MM1-5
Rat n 1	RUP-6/RUP-1	EPC-RN1-1	EPC-RN1-5
FOOD ALLERGENS			
Ana o 3	1H4/4A11	EPC-AO3-1	EPC-AO3-5
Api g 1	5d4/7C4	EPC-AG1-1	EPC-AG1-5
Ara h 1	2C12/2F7	EPC-AH1-1	EPC-AH1-5
Ara h 2	1C4/Rabbit anti Ara h 2	EPC-AH2-1	EPC-AH2-5
Ara h 3	1E8/4G9	EPC-AH3-1	EPC-AH3-5
Ara h 6	3B8/3E12	EPC-AH6-1	EPC-AH6-5
Ara h 8	4G6/Rabbit anti Ara h 8	EPC-AH8-1	EPC-AH8-5
Native Bos d 5 (β -lactoglobulin)	NBD5-1/NBD5-2	EPC-NBD5-1	EPC-NBD5-5
Bos d 11 (β -casein)	CC11/VB1C	EPC-BD11-1	EPC-BD11-5
Cor a 9	3B6/6F5	EPC-CA9-1	EPC-CA9-5
Gal d 1	5G11/2B2	EPC-GD1-1	EPC-GD1-5
Gal d 2	1B4/7D8	EPC-GD2-1	EPC-GD2-5
Gly m 5	6F6/1B9	EPC-GM5-1	EPC-GM5-5
Shrimp Tropomyosin	1A6/Rabbit anti Shrimp Tropomyosin	EPC-TPM-1	EPC-TPM-5
Sin a 1	3B4/2B11	EPC-SA1-1	EPC-SA1-5
MOLD ALLERGENS			
Alt a 1	2C10/3B6	EPC-AA1-1	EPC-AA1-5
Asp f 1	4A6/Rabbit anti Asp f 1	EPC-AF1-1	EPC-AF1-5
POLLEN ALLERGENS			
Amb a 1	2B6/4H7	EPC-AM1-1	EPC-AM1-5
Bet v 1	5B4/6H4	EPC-BV1-EP1	EPC-BV1-EP5
Cry j 1	4D12/2E11	EPC-CJ1-1	EPC-CJ1-5
Lol p 1	5G7/8D10	EPC-LP1-1	EPC-LP1-5
Phl p 5	1D11/Bo1	EPC-PP5-1	EPC-PP5-5