

ELISA 2.0

Quantitative Allergen Immunoassay Kit



- White Paper -

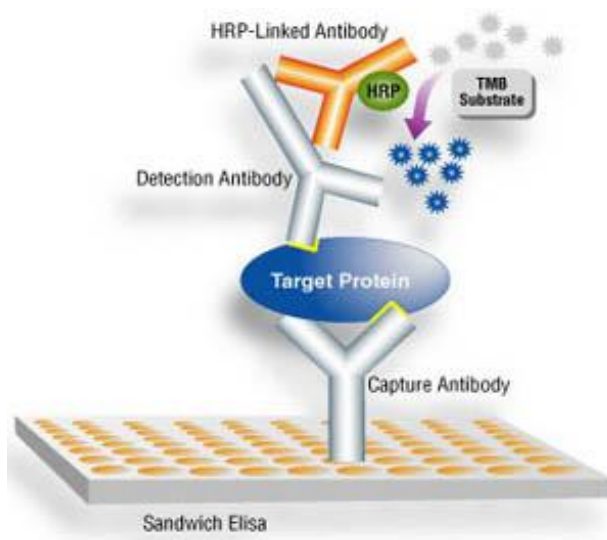
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Developed and manufactured by

INTRODUCTION

Enzyme Linked Immunosorbent Assay (ELISA) was developed in the early 1970s 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, InBio has built a comprehensive portfolio of proprietary, allergen-specific antibodies to allow the development of more than 40 different ELISA kits for the detection of dust mite, cockroach, mold, pollen, animal, and food allergens.

ELISA 2.0

InBio's 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.

- Pre-coated plates reduce the potential for operator error
- Validated using InBio's purified allergens
- Enhanced sensitivity and performance
- A complete list of ELISA 2.0 assays is shown in the Appendix on pages 7-8

ELISA 2.0 kits are available in single, five, and 25-plate format 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 InBio's ISO 9001:2015 certified laboratory and are validated using mass spectrometry, ELISA, and human IgE antibody binding.

ELISA 2.0 has been optimized to achieve maximum sensitivity and signal-to-noise ratio, while offering convenience and reproducible performance. Validated performance parameters for InBio's ELISA 2.0 kits for allergen measurement are provided in **Tables 1-5**.

Note: this product line is continually expanding; please visit our website at www.store.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 1a	Der p 2	Der p 23	Tyr p 2
Linearity (R²)¹	0.999	0.999	0.999	1	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	50-1.56	12.5-0.1	25-0.39	250-7.81
Limit of Quantification³								
LLOQ (ng/ml) ^{3a}	0.23-0.12	0.39-0.19	1.95-0.49	1.56-0.39	1.56-0.39	0.4-0.1	1.56-0.39	7.8-1.95
ULOQ (ng/ml) ^{3b}	30-7.5	25-12.5	250-62.5	50-25	50-6.25	25-6.25	25-12.5	500-250
Accuracy (% Recovery)⁴								
Intra-assay (n=9) ^{4a}	84-130%	88-118%	73-118%	98-117%	82-129%	83-132%	87-129%	93-116%
Inter-assay (n=54) ^{4b}	105%	106%	88%	103%	107%	104%	101%	108%
Precision (%CV)⁵								
Intra-assay (n=9) ^{5a}	1-16%	5-15%	1-12%	4-13%	1-23%	4-14%	3-11%	3-9%
Inter-assay (n=54) ^{5b}	8%	10%	7%	8%	11%	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	Ory c 3	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	1	0.999	0.999	0.999
Range (ng/ml)²	25-0.39	25-0.19	5-0.04	25-0.10	20-0.16	12.5-0.19	50-0.39	100-0.39	125-1.95	12.5-0.19
Limit of Quantification³										
LLOQ (ng/ml) ^{3a}	0.39	0.39-0.19	0.31-0.02	0.19-0.1	0.31-0.08	0.39-0.19	0.78-0.19	1.56-0.39	7.81-1.95	0.39-0.19
ULOQ (ng/ml) ^{3b}	25	25-12.5	5	25-12.5	20-5	50-25	50-25	50-25	250-125	50-6.25
Accuracy (% Recovery)⁴										
Intra-assay (n=9) ^{4a}	83-103%	96-129%	93-106%	91-113%	90-115%	85-129%	71-108%	92-113%	77-113%	71-110%
Inter-assay (n=54) ^{4b}	91%	113%	97%	103%	104%	102%	94%	101%	102%	99%
Precision (%CV)⁵										
Intra-assay (n=9) ^{5a}	5-18%	4-13%	4-8%	5-9%	2-14%	6-12%	3-9%	5-15%	4-12%	3-13%
Inter-assay (n=54) ^{5b}	9%	9%	6%	8%	6%	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							
LLOQ (ng/ml) ^{3a}	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
ULOQ (ng/ml) ^{3b}	25-6.25	40-20	200-25	100-50	50-25	250-125	250-62.5
Accuracy (% Recovery)⁴							
Intra-assay (n=9) ^{4a}	90-115%	78-115%	90-108%	82-120%	94-106%	81-112%	85-120%
Inter-assay (n=54) ^{4b}	99%	95%	100%	100%	100%	96%	103%
Precision (%CV)⁵							
Intra-assay (n=9) ^{5a}	3-11%	4-22%	6-12%	1-9%	6-16%	7-16%	7-10%
Inter-assay (n=54) ^{5b}	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	Jug r 1	Pru du 6
Linearity (R²)¹	1	0.999	0.999	1	1	1	0.998	0.997	0.999
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	25-0.39	125-0.49
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	0.78-0.39	3.91-0.49
<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	50-6.25	125-31.25
Accuracy (% Recovery)⁴									
<i>Intra-assay (n=9)^{4a}</i>	82-124%	80-116%	74-127%	105-113%	100-109%	85-110%	75-112%	86-119%	92-115%
<i>Inter-assay (n=54)^{4b}</i>	109%	101%	97%	108%	102%	99%	93%	108%	102%
Precision (%CV)⁵									
<i>Intra-assay (n=9)^{5a}</i>	3-12%	7-14%	3-11%	7-13%	3-15%	4-10%	5-15%	6-12%	4-15%
<i>Inter-assay (n=54)^{5b}</i>	8%	10%	5%	8%	8%	8%	10%	9%	7%

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

Allergen:	Api g 1	Bos d 5	Bos d 11	Cyp c 1	Gal d 1	Gal d 2	Gly m 5	Shrimp	Ses i 1	Sin a 1
Linearity (R²)¹	0.999	1	1	0.997	1	1	1	0.999	0.999	0.999
Range (ng/ml)²	100-1.56	12.5-0.1	500-7.81	62.5-0.98	250-7.81	50-0.39	250-1.95	25-0.20	25-0.20	15-0.06
Limit of Quantification³										
<i>LLOQ (ng/ml)^{3a}</i>	6.25-1.56	0.78-0.1	7.81-1.95	3.90-0.98	7.8-3.9	0.78-0.39	3.91-1.95	0.39-0.1	0.40-0.10	0.06-0.03
<i>ULOQ (ng/ml)^{3b}</i>	200-50	25-12.5	500-125	125-31.25	250	100-25	250-62.5	25-6.25	25-12.5	15-7.5
Accuracy (% Recovery)⁴										
<i>Intra-assay (n=9)^{4a}</i>	81-131%	84-120%	80-128%	71-120%	89-101%	91-132%	93-119%	95-115%	71-131%	70-132%
<i>Inter-assay (n=54)^{4b}</i>	108%	103%	94%	86%	95%	104%	105%	108%	109%	96%
Precision (%CV)⁵										
<i>Intra-assay (n=9)^{5a}</i>	3-11%	4-9%	2-16%	2-16%	8-13%	5-16%	5-11%	4-14%	3-27%	3-16%
<i>Inter-assay (n=54)^{5b}</i>	7%	6%	7%	7%	9%	11%	8%	7%	10%	8%

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

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 <http://www.inbio.com> to see InBio's 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

Faster 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



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

Appendix: Complete list of ELISA 2.0 assays (February 2024)

HOUSE DUST MITE ALLERGENS

Product Description	Antibody Pairs	Product Code		
		1 Plate	5 Plates	25 Plates
Blo t 5	Rabbit anti Blo t 5/ Rabbit anti Blo t 5	EPC-BT5-1	EPC-BT5-5	EPC-BT5-25
Der f 1	6A8/4C1	EPC-DF1-1	EPC-DF1-5	EPC-DF1-25
Der f 2	1D8/7A1	EPC-DF2-1	EPC-DF2-5	EPC-DF2-25
Der p 1	5H8/4C1	EPC-DP1-1	EPC-DP1-5	EPC-DP1-25
Der p 1a	10B9/5H8	EPC-DP1A-1	EPC-DP1A-5	EPC-DP1A-25
Der p 2	1D8/7A1	EPC-D2-1	EPC-D2-5	EPC-D2-25
Der p 23	Rabbit anti Der p 23/7A8	EPC-DP23-1	EPC-DP23-5	EPC-DP23-25

STORAGE MITE ALLERGENS

Product Description	Antibody Pairs	Product Code		
		1 Plate	5 Plates	25 Plates
Tyr p 2	Rabbit anti Tyr p 2/Rabbit anti Tyr p 2	EPC-TP2-1	EPC-TP2-5	EPC-TP2-25

COCKROACH ALLERGENS

Product Description	Antibody Pairs	Product Code		
		1 Plate	5 Plates	25 Plates
Bla g 1	10A6/Rabbit anti Bla g 1	EPC-BG1-1	EPC-BG1-5	EPC-BG1-25
Bla g 2	7C11/Rabbit anti Bla g 2	EPC-BG2-1	EPC-BG2-5	EPC-BG2-25
Bla g 5	17B12/Rabbit anti Bla g 5	EPC-BG5-1	EPC-BG5-5	EPC-BG5-25
Per a 7	Rabbit anti Shrimp Tropomyosin/1A6	EPC-PA7-1	EPC-PA7-5	EPC-PA7-25

ANIMAL ALLERGENS

Product Description	Antibody Pairs	Product Code		
		1 Plate	5 Plates	25 Plates
Can f 1 (Dog)	10D4/Rabbit anti Can f 1	EPC-CF1-1	EPC-CF1-5	EPC-CF1-25
Fel d 1 (Cat)	6F9/3E4	EPC-FD1-1	EPC-FD1-5	EPC-FD1-25
Fel d 4 (Cat)	5F3/Rabbit anti Fel d 4	EPC-FD4-1	EPC-FD4-5	EPC-FD4-25
Mus m 1 (Mouse)	Rabbit anti Mus m 1/Rabbit anti Mus m 1	EPC-MM1-1	EPC-MM1-5	EPC-MM1-25
Ory c 3 (Rabbit)	Goat anti Ory c 3/ Goat anti Ory c 3	EPC-OC3-1	EPC-OC3-5	EPC-OC3-25
Rat n 1 (Rat)	RUP-6/RUP-1	EPC-RN1-1	EPC-RN1-5	EPC-RN1-25

FOOD ALLERGENS

Product Description	Antibody Pairs	Product Code		
		1 Plate	5 Plates	25 Plates
Ana o 3 (Cashew)	1H4/4A11	EPC-AO3-1	EPC-AO3-5	EPC-AO3-25
Ara h 1 (Peanut)	2C12/2F7	EPC-AH1-1	EPC-AH1-5	EPC-AH1-25
Ara h 2 (Peanut)	1C4/Rabbit anti Ara h 2	EPC-AH2-1	EPC-AH2-5	EPC-AH2-25
Ara h 3 (Peanut)	1E8/4G9	EPC-AH3-1	EPC-AH3-5	EPC-AH3-25
Ara h 6 (Peanut)	3B8/3E12	EPC-AH6-1	EPC-AH6-5	EPC-AH6-25
Ara h 8 (Peanut)	4G6/Rabbit anti Ara h 8	EPC-AH8-1	EPC-AH8-5	EPC-AH8-25
Bos d 11 (Milk)	CC11/VBIC	EPC-BD11-1	EPC-BD11-5	EPC-BD11-25
Cor a 9 (Hazelnut)	3B6/6F5	EPC-CA9-1	EPC-CA9-5	EPC-CA9-25
Cyp c 1 (Fish)	9A6/7C5	EPC-CC-1	EPC-CC-5	EPC-CC-25
Gal d 1 (Egg)	5G11/2B2	EPC-GD1-1	EPC-GD1-5	EPC-GD1-25
Gal d 2 (Egg)	1B4/7D8	EPC-GD2-1	EPC-GD2-5	EPC-GD2-25
Gly m 4 (Soy)	1B10/Rabbit anti Gly m 4	EPC-GM4-1	EPC-GM4-5	EPC-GM4-25



FOOD ALLERGENS

Product Description	Antibody Pairs	Product Code		
		1 Plate	5 Plates	25 Plates
Gly m 5 (Soy)	6F6/1B9	EPC-GM5-1	EPC-GM5-5	EPC-GM5-25
Jug r 1 (Walnut)	7D7/Rabbit anti Jug r 1	EPC-JR1-1	EPC-JR1-5	EPC-JR1-25
Native Bos d 5 (Milk)	NBD5-1/NBD5-2	EPC-NBD5-1	EPC-NBD5-5	EPC-NBD5-25
Pru du 6 (Almond)	6C10/1D3	EPC-PD6-1	EPC-PD6-5	EPC-PD6-25
Shrimp Tropomyosin	1A6/Rabbit anti Shrimp Tropomyosin	EPC-TPM-1	EPC-TPM-5	EPC-TPM-25
Ses i 1 (Sesame)	Rabbit anti Ses i 1/Rabbit anti Ses i 1	EPC-SI1-1	EPC-SI1-5	EPC-SI1-25
Sin a 1 (Celery)	3B4/2B11	EPC-SA1-1	EPC-SA1-5	EPC-SA1-25

MOLD ALLERGENS

Product Description	Antibody Pairs	Product Code		
		1 Plate	5 Plates	25 Plates
Alt a 1	2C10/3B6	EPC-AA1-1	EPC-AA1-5	EPC-AA1-25
Asp f 1	4A6/Rabbit anti Asp f 1	EPC-AF1-1	EPC-AF1-5	EPC-AF1-25

POLLEN ALLERGENS

Product Description	Antibody Pairs	Product Code		
		1 Plate	5 Plates	25 Plates
Amb a 1 (Ragweed)	2B6/4H7	EPC-AM1-1	EPC-AM1-5	EPC-AM1-25
Bet v 1 EP (Birch)	5B4/6H4	EPC-BV1-EP1	EPC-BV1-EP5	EPC-BV1-EP25
Cry j 1 (Japanese Cedar)	4D12/2E11	EPC-CJ1-1	EPC-CJ1-5	EPC-CJ1-25
Lol p 1 (Ryegrass)	5G7/8D10	EPC-LP1-1	EPC-LP1-5	EPC-LP1-25
Phl p 5 (Timothy grass)	1D11/Bo1	EPC-PP5-1	EPC-PP5-5	EPC-PP5-25

