

ELISA 2.0

Quantitative Allergen Immunoassay Kit

- White Paper -

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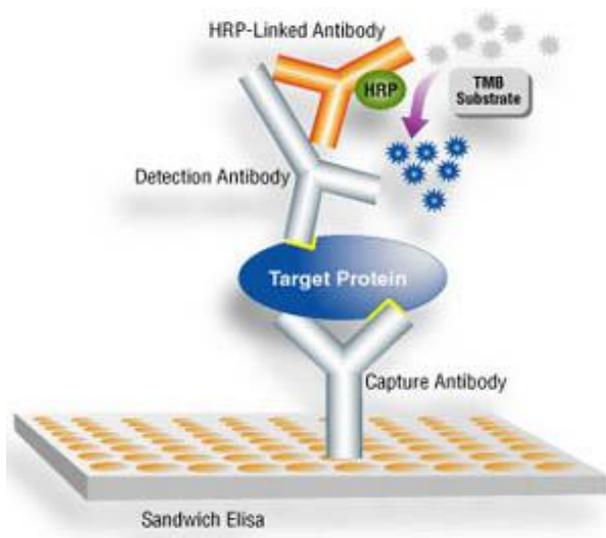
Developed and Manufactured by



INTRODUCTION

Enzyme Linked Immunosorbent Assay (ELISA) is a method used to measure an analyte by means of an immunological reaction. ELISA was developed in the early 1970's as a safe alternative to radioimmunoassays. Because of the relative ease of use, potential for high throughput screening, and readily available reagents, ELISA has become a standard technique used throughout the world with applications ranging from HIV screening to environmental allergen 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 20 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 kits are used 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 over 35 different countries. These traditional ELISA kits contain all essential components needed to perform the assay; however, non-assay-specific reagents and buffers are also required. These additional materials are available from a range of different vendors and can vary in quality. Traditional ELISA assays also require an overnight incubation step in addition to the remaining assay time.

To improve overall assay performance and operator convenience, Indoor Biotechnologies recognized the need to offer complete ELISA kits: ELISA 2.0.

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, and include the following:

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



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

How is that possible? Pre-coated plates eliminate the overnight incubation step and the need to plan experiments a day 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, several ELISA 2.0 assays also offer enhanced sensitivity compared to traditional ELISA. Individual ELISA 2.0 assay validation reports are available upon request.

A summary of ELISA 2.0 performance characteristics are presented in Table I and Table II.

Table I. ELISA 2.0 Performance Characteristics

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
Linearity (R²)¹	1	0.999	0.999	1	1	1	0.999	1
Range (ng/ml)²	100-0.78	25-0.39	25-0.20	25-0.39	25-0.10	12.5-0.20	100-0.39	250-0.98
Limit of Quantification³								
<i>LLOQ (ng/ml)^{3a}</i>	1.56-0.39	0.39-0.20	0.39-0.20	0.39	0.20-0.10	0.39-0.20	1.56-0.39	1.95-0.98
<i>ULOQ (ng/ml)^{3b}</i>	50-25	25-12.5	25-12.5	25	25-12.5	50-25	50-25	250-62.5
Accuracy (% Recovery)⁴								
<i>Intra-assay (n=9)^{4a}</i>	98-117%	88-118%	100-129%	83-103%	91-113%	85-129%	92-113%	85-120%
<i>Inter-assay (n=54)^{4b}</i>	103%	106%	113%	91%	103%	102%	101%	103%
Precision (%CV)⁵								
<i>Intra-assay (n=9)^{5a}</i>	4-13%	5-15%	4-13%	5-18%	5-9%	6-12%	5-15%	7-10%
<i>Inter-assay (n=54)^{5b}</i>	8%	10%	9%	9%	8%	10%	10%	9%

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 II. 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
ELISA 2.0	0.39	0.20	0.20	0.39	0.10	0.20	0.39	0.98
Traditional ELISA	0.98	0.98	0.39	0.98	0.39	0.39	0.98	0.98

LLOQ values (ng/ml)

SUMMARY

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

Visit www.inbio.com to see Indoor Biotechnologies' expanding range of ELISA 2.0 kits.

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