

ELISA 2.0 - Validated Performance Parameters

Food Allergens:

Allergen:	Ana o 3	Ara h 1	Ara h 2	Ara h 3	Ara h 6	Ara h 8	Api g 1	Bos d 5 (Native)	Bos d 11	Cor a 9
Linearity (R²)¹	1.0	1.0	0.999	0.999	1.0	1.0	0.999	1.0	1.0	0.998
Range (ng/ml)²	20-0.16	1000-31.25	125-0.98	62.5-0.49	25.0-0.05	12.5-0.20	100-1.56	12.5-0.010	500-7.81	50-0.39
Limit of Quantification³										
<i>LLOQ (ng/ml)^{3a}</i>	0.16	15.63-31.25	0.49-3.91	0.49-1.95	0.05-0.20	0.20-1.56	1.56-6.25	0.10-0.78	1.95-7.81	0.39-0.78
<i>ULOQ (ng/ml)^{3b}</i>	20-5	1000	250-31.25	62.5-31.25	25-12.5	25-6.25	200-50	25-12.5	500-125	100-12.5
Accuracy (% Recovery)⁴										
<i>Intra-assay (n=9)^{4a}</i>	85-110%	77-128%	80-116%	74-127%	105-113%	100-109%	81-130%	84-120%	75-128%	75-112%
<i>Inter-assay (n=36)^{4b}</i>	99%	109%	101%	97%	108%	102%	108%	103%	94%	93%
Precision (%CV)⁵										
<i>Intra-assay (n=9)^{5a}</i>	4-10%	2-19%	7-14%	3-11%	7-13%	3-15%	3-11%	4-9%	2-16%	5-15%
<i>Inter-assay (n=36)^{5b}</i>	8%	8%	10%	5%	8%	8%	7%	6%	7%	10%

Allergen:	Cyp c 1	Gal d 1	Gal d 2	Gly m 5	Jug r 1	Pru du 6	Ses i 1	Shrimp Tropomyosin	Sin a 1
Linearity (R²)¹	0.997	1.0	1.0	1.0	0.997	0.999	0.999	0.999	0.999
Range (ng/ml)²	62.5-0.98	250-7.81	50-0.39	250-1.95	25-0.39	125-0.49	25-0.20	25-0.20	15-0.06
Limit of Quantification³									
<i>LLOQ (ng/ml)^{3a}</i>	0.98-3.90	3.90-7.80	0.39-0.78	1.95-3.91	0.40-0.78	0.49-3.91	0.10-0.40	0.10-0.39	0.03-0.06
<i>ULOQ (ng/ml)^{3b}</i>	125-31.25	250	100-25	250-62.5	50-6.25	125-31.25	25-12.5	25-6.25	15-7.5
Accuracy (% Recovery)⁴									
<i>Intra-assay (n=9)^{4a}</i>	71-120%	89-101%	91-132%	93-119%	86-119%	92-115%	71-131%	95-115%	70-132%
<i>Inter-assay (n=36)^{4b}</i>	86%	95%	104%	105%	108%	102%	109%	108%	96%
Precision (%CV)⁵									
<i>Intra-assay (n=9)^{5a}</i>	2-16%	8-13%	5-16%	5-11%	6-12%	4-15%	3-27%	4-14%	3-16%
<i>Inter-assay (n=36)^{5b}</i>	7%	9%	11%	8%	9%	7%	10%	7%	8%

Validation Notes:

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**
 - 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**
 - 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**
 - 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).