700 Harris Street
Charlottesville, VA
22903
U.S.A.

Tel: (1) (434) 984-2304
Fax: (1) (434) 984-2709
www.inbio.com

ELISA 2.0 Inhalable Allergens – Validated Performance Parameters

	HOUSE DUST MITE								STORAGE MITES
Allergen:	**Der p 1**	**Der p 1a**	**Der p 23**	**Der p 2**	**Der f 1**	**Der f 2**	**Blo t 5**	**Eur m 1**	**Tyr p 2**
Linearity (R^2)[1]	1.0	1.0	0.998	0.999	0.999	0.999	0.999	1.0	0.999
Range (ng/ml)[2]	100-0.78	50-1.56	50-0.10	12.5-0.10	25-0.39	62.5-0.98	7.5-0.12	60-0.23	250-7.81
Limit of Quantification[3]									
LLOQ (ng/ml)[3a]	0.39-1.56	0.39-1.56	0.391.56	0.10-0.40	0.20-0.39	0.49-1.95	0.12-0.23	0.12-0.94	1.95-7.80
ULOQ (ng/ml)[3b]	50-25	50-6.25	25-12.5	25-6.25	25-12.5	250-62.5	30-3.75	60-15	500-250
Accuracy (% Recovery)[4]									
Intra-assay (n=9)[4a]	98-117%	88-122%	92-112%	83-132%	88-118%	83-97%	85-120%	92-104%	93-116%
Inter-assay (n=54)[4b]	103%	107%	101%	104%	106%	88%	105%	100%	108%
Precision (%CV)[5]									
Intra-assay (n=9)[5a]	4-13%	7-16%	4-15%	4-14%	5-15%	6-7%	3-12%	3-8%	3-9%
Inter-assay (n=54)[5b]	8%	11%	8%	7%	10%	6%	8%	6%	6%

	ANIMAL							COCKROACH			
Allergen:	**Fel d 1**	**Fel d 4**	**Can f 1**	**Mus m 1**	**Ory c 3**	**Rat n 1**	**Equ c 1**	**Bla g 1**	**Bla g 2**	**Bla g 5**	**Per a 7**
Linearity (R^2)[1]	0.999	1.0	1.0	1.0	0.999	1.0	0.999	1.0	0.999	0.999	0.999
Range (ng/ml)[2]	25-0.20	5-0.04	25-0.39	25-0.10	20-0.16	50-0.39	62.5-0.98	50-0.39	100-0.39	125-1.95	12.5-0.20
Limit of Quantification[3]											
LLOQ (ng/ml)[3a]	0.20-0.39	0.02-0.31	0.39	0.10-0.20	0.08-0.31	0.20-0.39	0.49-0.98	0.20-0.78	0.39-1.56	0.98-7.81	0.19-0.39
ULOQ (ng/ml)[3b]	25-12.5	5	25	25-12.5	20-5	50-25	125-62.5	50-25	50-25	250-125	50-6.25
Accuracy (% Recovery)[4]											
Intra-assay (n=9)[4a]	96-129%	93-106%	83-103%	91-113%	92-116%	85-129%	90-105%	71-108%	92-113%	77-111%	71-110%
Inter-assay (n=54)[4b]	113%	97%	91%	103%	104%	102%	100%	94%	101%	102%	99%
Precision (%CV)[5]											
Intra-assay (n=9)[5a]	4-13%	4-8%	5-18%	5-9%	4-12%	6-12%	6-13%	3-9%	5-15%	4-12%	3-13%
Inter-assay (n=54)[5b]	9%	6%	9%	8%	6%	9%	9%	6%	10%	8%	7%

700 Harris Street
Charlottesville, VA
22903
U.S.A.

Tel: (1) (434) 984-2304
Fax: (1) (434) 984-2709
www.inbio.com

Allergen:	POLLENS					MOLDS		CANNABIS		VENOM		
	Bet v 1 EP	Phl p 5	Amb a 1	Lol p 1	Cry j 1	Alt a 1	Asp f 1	Can s 3	Can s 5	Bom t 1	Ves v 2	Ves v 5
Linearity (R^2)[1]	0.999	1.0	1.0	0.994	1.0	1.0	1.0	1.0	1.0	0.999	0.997	0.994
Range (ng/ml)[2]	50-0.39	250-0.98	100-0.78	125-7.81	50-0.78	25-0.10	40-0.31	62.5-0.49	25-0.39	2.5-0.08	25-1.56	12.5-0.78
Limit of Quantification[3]												
LLOQ (ng/ml)[3a]	0.39	1.95-0.98	1.56-0.78	15.63-3.91	0.78-0.39	0.40-0.10	0.16-0.63	0.24-0.49	0.10-0.39	0.08-0.16	0.78-3.13	0.39-0.78
ULOQ (ng/ml)[3b]	100-50	250-62.5	200-25	250-125	50-25	25-6.25	40-20	62.5	25-50	5-10	25-50	12.5-50
Accuracy (% Recovery)[4]												
Intra-assay (n=9)[4a]	82-120%	85-120%	90-108%	81-111%	94-106%	90-115%	78-115%	100-115%	90-99%	98-107%	91-112%	95-120%
Inter-assay (n=54)[4b]	100%	102%	100%	96%	100%	99%	95%	105%	93%	101%	102%	110%
Precision (%CV)[5]												
Intra-assay (n=9)[5a]	1-9%	7-10%	6-12%	7-16%	5-16%	3-11%	4-22%	5-14%	4-11%	6-15%	3-14%	5-11%
Inter-assay (n=54)[5b]	7%	9%	8%	12%	10%	7%	12%	11%	7%	10%	8%	8%

700 Harris Street
Charlottesville, VA
22903
U.S.A.

Tel: (1) (434) 984-2304
Fax: (1) (434) 984-2709
www.inbio.com

ELISA 2.0 Food Allergens – Validated Performance Parameters

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 (R^2)[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	25.0.05	12.5-0.20	100-1.56	12.5-0.010	500-7.81	50-0.39
Limit of Quantification[3]										
LLOQ (ng/ml)[3a]	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
ULOQ (ng/ml)[3b]	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]										
Intra-assay (n=9)[4a]	85-110%	82-124%	80-116%	74-127%	105-113%	100-109%	96-119%	84-120%	83-123%	75-112%
Inter-assay (n=54)[4b]	99%	109%	101%	97%	108%	102%	108%	103%	94%	93%
Precision (%CV)[5]										
Intra-assay (n=9)[5a]	4-10%	3-12%	7-14%	3-11%	7-13%	3-15%	5-9%	4-9%	5-11%	5-15%
Inter-assay (n=54)[5b]	8%	8%	10%	5%	9%	8%	7%	6%	7%	10%

Allergen:	Cyp c 1	Gad m 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 (R^2)[1]	0.997	0.999	1.0	1.0	1.0	0.997	0.999	0.999	0.999	0.999
Range (ng/ml)[2]	62.5-0.98	50-0.39	250-7.81	50-0.39	250-1.95	25-0.39	125-0.49	25-0.20	25-0.20	100-0.39
Limit of Quantification[3]										
LLOQ (ng/ml)[3a]	0.98-3.90	0.369-1.56	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	0.39-0.78
ULOQ (ng/ml)[3b]	125-31.25	50-25	250	100-25	250-62.5	50-6.25	125-31.25	25-12.5	25-6.25	100-12.5
Accuracy (% Recovery)[4]										
Intra-assay (n=9)[4a]	74-110%	87-112%	95-107%	91-132%	93-119%	104-110%	92-109%	80-124%	95-115%	98-121%
Inter-assay (n=54)[4b]	86%	102%	95%	103%	105%	108%	102%	108%	108%	108%
Precision (%CV)[5]										
Intra-assay (n=9)[5a]	4-11%	2-20%	4-10%	5-16%	5-11%	8-11%	3-10%	8-14%	4-14%	3-11%
Inter-assay (n=54)[5b]	7%	7%	9%	11%	8%	9%	6%	10%	7%	6%

700 Harris Street
Charlottesville, VA
22903
U.S.A.

Tel: (1) (434) 984-2304
Fax: (1) (434) 984-2709
www.inbio.com

Validation Notes:

1. **Linearity** is the mean R^2 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). Equ c 1 sample A (n=4).
   - 4b. Inter-assay - The overall average percent recovery of samples A, B, and C run in triplicate from six ELISA plates (n=54). Equ c 1 sample A (n=24).

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). Equ c 1 sample A (n=4).
   - 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). Equ c 1 sample A (n=24).

6. Bet v 1 ELISA 2.0 EP has been cross-validated against the candidate Ph. Eur. Method for Bet v 1 determination.