

# Animal samples in Mercodia Glucagon ELISA - 10 $\mu$ L (10-1281-01)

Having the right research models, as well as the tools to match them, is essential for making new scientific advances. Mercodia Glucagon ELISA -10  $\mu$ L is a unique tool for investigating glucagon secretion. The assay, which is fully validated for rat, mouse and non-human primate samples, can also be used for dog, pig, sheep and cat samples.

## Scope of the technical note

This Technical Note presents performance characteristics for dog and pig samples and lot-to-lot variability generated at Mercodia. In addition, it summarizes work performed for cat and sheep samples off-site that has been peer-reviewed and published in scientific journals.

## Performance characteristics for dog and pig samples

The validation studies performed for dog and pig samples were acceptable according to pre-determined criteria.

## Precision

Within-assay variation was less than 8% for both species, which was well within the acceptance criteria of 15%. Total assay variation was less than 16%, which was also acceptable ( $\leq 25\%$ ), see Table 1.

## Dilution

The results from the dilution study were acceptable (O/E less than  $\pm 25\%$ ), see Table 2 and Table 3.



Table 1. At least 8 runs with 4 sample replicates in one assay lot.

Species	Mean (pmol/L)	Repeatability %*	Within laboratory %**
Dog	5.89	7.2	12.6
	7.81	7.4	15.7
	13.0	6.9	10.5
Pig	5.43	6.7	12.9
	5.86	6.4	13.9
	13.2	7.7	8.3

\* Within assay CV

\*\* Total assay CV

Table 2. Seven dog samples diluted 1/2 and 1/4. Mean recovery was 100% (87% - 124%).

Sample ID	Dilution Factor	Mean glucagon (pmol/L)	O/E E=neat sample
MS-4339	1	5.9	
	2	3.1	103%
	4	1.9	124%
MS-4341	1	11.1	
	2	5.2	94%
	4	3.0	107%
MS-4342	1	8.2	
	2	3.9	95%
	4	2.2	105%
MS-4344	1	7.6	
	2	3.4	90%
	4	1.9	102%
MS-4350	1	8.5	
	2	4.1	96%
	4	2.1	100%
MS-4352	1	4.7	
	2	2.0	87%
	4	<Min	-
MS-4353	1	5.2	
	2	2.4	91%
	4	<Min	-

Table 3. Nine pig samples diluted 1/2 and 1/3. Mean recovery was 87% (74% - 114%).

Sample ID	Dilution Factor	Mean glucagon level (pmol/L)	O/E E=neat sample
MS-4422	1	8.0	
	2	3.0	76%
	3	<Min	-
MS-4423	1	8.4	
	2	3.7	87%
	3	2.3	83%
MS-4424	1	5.1	
	2	2.3	92%
	3	1.9	114%
MS-4425	1	10.3	
	2	4.5	87%
	3	2.6	74%
MS-4426	1	6.8	
	2	2.9	84%
	3	<Min	-
MS-4428	1	9.5	
	2	4.9	104%
	3	2.6	83%
MS-4429	1	8.6	
	2	4.1	95%
	3	2.4	83%
MS-4432	1	8.5	
	2	3.6	85%
	3	2.1	75%
MS-4436	1	9.6	
	2	4.2	88%
	3	2.8	88%

### Lot-to-lot variability for pig samples

The stringent quality system employed by Merckodia did not accept the lot-to-lot variability seen for porcine samples in Merckodia Glucagon ELISA - 10 µL. The correlation between lots was acceptable ( $R^2 = 0.85$ ) but not the absolute values (average O/E = 137%). Figure 1 and Table 4 show the results.

Merckodia will reserve specific assay lots upon request and recommends using this service when species other than mouse, rat or non-human primates are to be studied with Merckodia Glucagon ELISA - 10 µL. This is also in line with regulatory guidelines that recommend using the same study conditions for all samples in an investigation to avoid introducing unnecessary errors.

Please contact Merckodia for more information at [info-global@merckodia.com](mailto:info-global@merckodia.com).

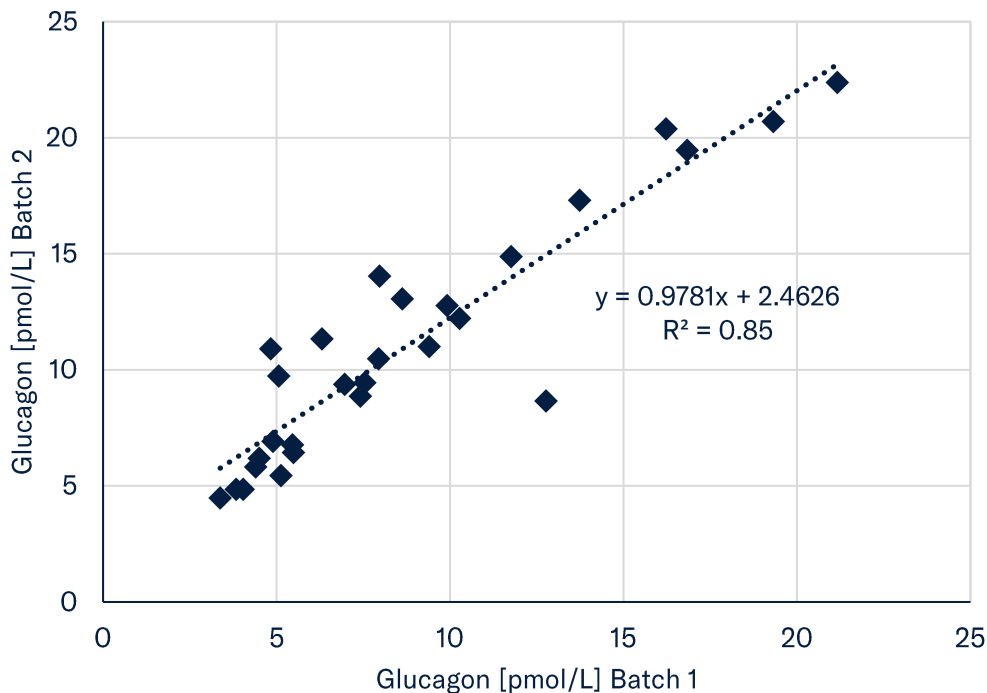


Figure 1. Glucagon levels (pmol/L) measured in pig samples (n=24) with two different kit lots.  $R^2 = 0.85$ .

Table 4. Lot-to-lot comparison using pig samples (n=24) with two kit lots of Merckodia Glucagon ELISA - 10 µL.

Number of samples	Correlation coefficient	Average O/E	Standard deviation of the mean
n = 24	$R^2 = 0.85$	137%	±32%

### Validation of cat samples

Validation of feline samples in the Mercodia Glucagon ELISA – 10 µL (10-1281-01) has been published in the journal of Domestic Animal Endocrinology by Rudinsky and co-authors<sup>1</sup>. Healthy, 3-years old castrated male cats were used in the study. The authors report that linear regression for expected vs observed results in serial dilutions were  $R^2 = 0.9958$  ( $P < 0.0001$ ). Moreover, the variation and recovery described in the publication are summarized in table 5.

### Validation of sheep samples

Validation of the Mercodia Glucagon ELISA - 10 µL (10-1281-01) has been published in the Journal of Endocrinology by Adhikari and co-authors<sup>2</sup>. The validation for sheep plasma included dilution, spiking and evaluation of intra- and inter-assay coefficients of variation for high and low glucagon control samples and the results are summarized in table 6.

Table 5. Summary of validation data reported in Rudinsky et al, 2015<sup>1</sup>. Feline sample validation

	CV (%)	Recovery (%)
1.5 – 3 pg/mL	7	-
8 – 15 pg/mL	4.6	-
Intra-assay variation	6.7	-
Inter-assay variation	8.1	-
Spiking	-	86.8 - 102

Table 6. Summary of validation data reported in Adhikari et al, 2018<sup>2</sup>. Ovine sample validation

	CV (%)	Recovery (%)
Intra-assay variation	5.32 / 7.67	-
Inter-assay variation	8.22 / 9.15	-
Spiking	-	78 ± 0.8
2-fold dilution	-	89 ± 6.01

**References**

1. Rudinsky, A. J. *et al.* Pharmacology of the glucagon-like peptide-1 analog exenatide extended-release in healthy cats. *Domest Anim Endocrinol* **51**, 78–85 (2015).
2. Adhikari, B., Khanal, P. & Nielsen, M. O. Impacts of pre- and postnatal nutrition on glucagon regulation and hepatic signalling in sheep. *J. Endocrinol.* JOE-17-0705 (2018). doi:10.1530/JOE-17-0705