Mercodia
Developing diagnostics

Technical Note No: 34-0134

Cross-reactivity in Rat and Mouse Insulin ELISAs

When cross-reactivity data is interpreted, the physiological concentrations should always be considered.

Some studies suggest that the physiological circulating proinsulin concentration is approximately 5-20 % of the insulin concentration (J. A Ehses *et al.*, 2009; P. Lebrun *et al.*, 2010). This is confirmed by in-house studies with normal Sprague Dawley rats. These studies showed a proinsulin concentration range of 12 to 71.5 pmol/L (0.11-0.65 µg/L) with a median value of 24 pmol/L (0.2 µg/L). The insulin concentration for the same samples ranged from 0.63 to 2.2 µg/L with a median value of 1.8 µg/L. It should be noted that these values (% and concentration) may vary based on research model used (e.g. mouse, rat, health status, type 1 diabetic, insulin-resistant, etc.).

The ratio between proinsulin I and II is approximately 1.5 in rats and 0.5 in mice (S. Linde *et al.*, 1993).

If elevated proinsulin concentrations are expected, which may significantly contribute to the response in the insulin assay, combined analysis of rodent proinsulin and insulin are recommended. This can be done with the Mercodia Rat/Mouse Proinsulin ELISA and one of Mercodia's rodent Insulin ELISAs. The measured concentration in the proinsulin assay should be multiplied with the cross-reactivity in the insulin assay and subtracted from the measured insulin concentration.

For example:
A rat sample was determined to have an insulin concentration of 1.8 µg/L (using the Mercodia Rat Insulin ELISA) and a proinsulin concentration of 0.2 µg/L (using the Mercodia Rat/Mouse Proinsulin ELISA). Based on publications, the ratio between proinsulin I and proinsulin II in rats is approximately 1.5, which in this case results in 0.12 µg/L proinsulin I and 0.08 µg/L proinsulin II according to the following calculations:

$$0.2\mu g/L\ proinsulin = 3\ parts\ proinsulinI + 2\ parts\ proinsulinII$$
$$\Rightarrow 1\ part = 0.04\mu g/L$$

The cross reactivity of proinsulin I is 8% and proinsulin II is 51 % in the Mercodia Rat Insulin ELISA. Hence, the contribution of proinsulin to the measured insulin value is 0.05 µg/L or 2.8%.

$$8\% \times 0.12\mu g/L + 51\% \times 0.08\mu g/L = 0.05\mu g/L$$

or

$$\frac{0.05}{1.8} \times 100 = 2.8\%$$

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Specificity of Rat Insulin ELISA

The following cross-reactions have been found:

Rat Proinsulin I	8%
Rat Proinsulin II	51%
Mouse Proinsulin I	33%
Mouse Proinsulin II	51%
Mouse C-peptide I	< 0.002%
Mouse C-peptide II	< 0.001%
Mouse Insulin	75%
Rat C-peptide I	<0.03%
Rat C-peptide II	<0.03%
Human Insulin	167%
Human C-peptide	<0.05%
Human proinsulin	75%

Specificity of Mouse Insulin ELISA

The following cross-reactions have been found:

Rat Proinsulin I	14%
Rat Proinsulin II	60%
Mouse Proinsulin I	43%
Mouse Proinsulin II	60%
Mouse C-peptide I	< 0.002%
Mouse C-peptide II	< 0.002%
Rat Insulin	146%
Rat C-peptide I	<0.04%
Rat C-peptide II	<0.04%
Human Insulin	195%
Human C-peptide	<0.05%
Human proinsulin	82%

The cross-reactivities were determined using purified recombinant proteins.

References

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