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 Ehres 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:

\[
0.2 \mu g/L \text{ proinsulin} = 3 \text{ parts proinsulin I} + 2 \text{ parts proinsulin II}
\]
\[
\Rightarrow 1 \text{ part} = 0.04 \mu g/L
\]

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
\[
\frac{0.05}{1.8} \times 100 = 2.8\%
\]
**Technical Note**

**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**

