Mercodia Glucagon ELISAs

Bibliography
Glucagon ELISA (article no. 10-1271-01)


Malmgren S, Ahren B. DPP-4 inhibition contributes to the prevention of hypoglycaemia through a GIP-glucagon counterregulatory axis in mice. Diabetologia. 2015.


Glucagon ELISA - 10 µL (article no. 10-1281-01)


MERCODIA GLUCAGON ELISA
article no. 10-1271-01

Intended use
A high quality enzyme immunoassay for the quantification of glucagon in serum, EDTA plasma, P800 stabilized samples and cell culture medium.

Test principle
Mercodia Glucagon ELISA is a solid phase two-site enzyme immunoassay based on the sandwich technique, in which two monoclonal antibodies are directed against separate antigenic determinants on the glucagon molecule. Glucagon in the sample reacts with anti-glucagon antibodies bound to microtitration wells and peroxidase-conjugated anti-glucagon antibodies in the solution.

Test characteristics

Sensitivity
The detection limit is 1 pmol/L as determined with the methodology described in ISO11843- Part 4.

Recovery
Recovery upon addition 96–101% (Mean 98%)
Recovery upon dilution 81–96% (Mean 86%)

Precision
Each sample was analyzed in 4 replicates on 39 different occasions.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean value (pmol/L)</th>
<th>within assay %</th>
<th>between assay %</th>
<th>total assay %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.0</td>
<td>5.1</td>
<td>8.1</td>
<td>8.5</td>
</tr>
<tr>
<td>2</td>
<td>5.2</td>
<td>3.6</td>
<td>9.4</td>
<td>9.5</td>
</tr>
<tr>
<td>3</td>
<td>21.9</td>
<td>3.3</td>
<td>7.3</td>
<td>7.5</td>
</tr>
</tbody>
</table>

Performance limitations
Grossly lipemic, icteric or haemolyzed samples do not interfere in the assay.

Specificity
The following cross-reactions have been tested:

- Glicentin <0.8%
- Oxyntomodulin <4.4%
- Mini-glucagon <0.10%
- GRPP <0.0005%
- GLP-1 (1-37) <0.30%
- GLP-1 (1-36 amide) <0.30%
- GLP-1 (9-36 amide) <0.30%
- GLP-1 (7-37) <0.30%
- GLP-2 <0.30%

Samples
Serum, EDTA plasma and cell culture medium samples can be used. Currently validated for human, rat and mouse samples. For other species, please contact Mercodia.

Measurement range
15 - 120 pmol/L (5 - 414 pg/mL)

Catalog No
10-1271-01  1 x 96 wells

Summary of protocol
- Add 25 µL Calibrators, controls and samples
- Add 200 µL enzyme conjugate 1X solution
- Incubate 18-22 h (overnight) on shaker at 2–8°C
- Wash plate 6 times
- Add 200 µL Substrate TMB
- Incubate 15 minutes at room temperature
- Add 50 µL Stop Solution
  Shake for approximately 5 seconds on shaker
- Measure at 450 nm
Intended use
A high quality enzyme immunoassay for the quantitative determination of glucagon in rat, mouse, porcine and non-human primate (NHP) samples.

Test principle
Mercodia Glucagon ELISA – 10 µL is a solid phase two-site enzyme immunoassay based on the sandwich technique, in which two monoclonal antibodies are directed against separate antigenic determinants on the glucagon molecule. Glucagon in the sample reacts with anti-glucagon antibodies bound to microtitration wells and peroxidase-conjugated anti-glucagon antibodies in the solution.

Test characteristics
Sensitivity
The detection limit is 1.5 pmol/L (5.2 pg/mL) as determined with the methodology described in ISO11843- Part 4.

Recovery

<table>
<thead>
<tr>
<th>Species</th>
<th>Addition Min</th>
<th>Addition Max</th>
<th>Addition Mean</th>
<th>Dilution Min</th>
<th>Dilution Max</th>
<th>Dilution Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Porcine</td>
<td>101</td>
<td>118</td>
<td>111</td>
<td>74</td>
<td>114</td>
<td>87</td>
</tr>
<tr>
<td>NHP*</td>
<td>96</td>
<td>112</td>
<td>103</td>
<td>85</td>
<td>106</td>
<td>95</td>
</tr>
<tr>
<td>Mouse</td>
<td>109</td>
<td>126</td>
<td>118</td>
<td>78</td>
<td>101</td>
<td>85</td>
</tr>
<tr>
<td>Rat</td>
<td>114</td>
<td>121</td>
<td>118</td>
<td>71</td>
<td>125</td>
<td>92</td>
</tr>
</tbody>
</table>

Figures in %
*Non-human primate samples kindly provided by Professor Barbara C. Hansen at University of South Florida, USA.

Precision
Each sample was analyzed in 4 replicates on at least 7 different occasions.

<table>
<thead>
<tr>
<th>Species</th>
<th>Sample</th>
<th>Mean value pmol/L</th>
<th>Coefficient of variation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Repeatability %*</td>
<td>Within laboratory %**</td>
</tr>
<tr>
<td>Porcine</td>
<td>1</td>
<td>5.4</td>
<td>6.7</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>5.9</td>
<td>6.4</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>13.2</td>
<td>7.7</td>
</tr>
<tr>
<td>NHP</td>
<td>1</td>
<td>12.6</td>
<td>7.2</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>36.9</td>
<td>7.4</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>44.7</td>
<td>7.7</td>
</tr>
<tr>
<td>Rat</td>
<td>1</td>
<td>7.3</td>
<td>5.1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>8.8</td>
<td>6.9</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>9.6</td>
<td>4.4</td>
</tr>
</tbody>
</table>

* Within assay variation
** Total assay variation

Performance limitations
Grossly lipemic or icteric samples do not interfere in the assay. Samples with high levels of hemoglobin (>500 mg/dl) can interfere in the assay.

Summary of protocol
- Add 10 µL Calibrators, controls and samples
- Add 50 µL enzyme conjugate 1X solution
- Incubate 18-22 h (overnight) on shaker at 2-8°C
- Wash plate 6 times
- Add 200 µL Substrate TMB
- Incubate 15 minutes at room temperature
- Add 50 µL Stop Solution
  - Shake for approximately 5 seconds on shaker
  - Measure at 450 nm

Samples
Serum, EDTA plasma and cell culture medium samples can be used.

Measurement range
2-180 pmol/L (7 - 627 pg/mL)

Catalog No
10-1281-01 1 x 96 wells

Specificity
- Mini-glucagon n.d.
- Glicentin, human 1.0%
- Glicentin, mouse 7.0%
- Glicentin, rat 4.0%
- Oxyntomodulin, human/rat/mouse 2.0%
- Oxyntomodulin, bovine/canine/porcine n.d.
- GLP-1 (7-36) n.d.
- GLP-1 (9-36) n.d.
- GLP-2 n.d.
Mercodia AB is a Swedish biotech company focusing on the development of immunoassays for research within the field of metabolic disorders. Our assays are applicable to both animal and human models and are used for research ranging from basic scientific studies to large pre-clinical and clinical phase trials.

The company was founded in 1991 and is today a world-leading supplier of products to all major international markets.

More than ninety percent of our production is exported from our facilities in Uppsala to approximately 100 different countries around the world.

Mercodia provides a professional scientific support system by collaborating with customers and institutions worldwide to develop novel applications for existing products and unique diagnostics for emerging markets.