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UC Davis - Glucagon V.2

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Peter Havel[1]

[1]University of California, Davis

Mouse Metabolic Phenotyping Centers
Tech. support email: **info@mmpc.org**


Lili Liang

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We use this protocol and it's working

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Abstract

Summary:

Glucagon is a 29 amino acid polypeptide processed from proglucagon in pancreatic alpha cells. In intestinal L-cells proglucagon is cleaved into glicentin, corresponding to proglucagon residues no 1-69. Glicentin can further be processed into oxyntomodulin, corresponding to proglucagon residues no 33-69. These peptides are released simultaneously upon stimulation. Moreover, a fragment of glucagon corresponding to its Cterminal part (residues no 19-29), also designated mini-glucagon, is reported to be present in the pancreas in low amounts compared to the total glucagon content.
In general, glucagon has an effect opposite that of insulin, i.e. it raises blood glucose levels. It causes the liver to convert glycogen into glucose, which is then released into the blood stream. With longer stimulation, glucagon action in the liver results in a glucose-sparing activation of free fatty acid oxidation and production of ketones. During hypoglycaemia, glucagon secretion offers a protective feedback mechanism, defending the organism against damaging effects of glucose deficiency in the brain and nerves.

Materials

MATERIALS

Glucagon ELISA **Mercodia Catalog #**10-1281-01

Reagent Preparation:

Antibody:
Prepare the needed volume of enzyme conjugate 1X solution by dilution of Enzyme Conjugate 11X (1+10) in Enzyme Conjugate Buffer according to the table below. When preparing enzyme conjugate 1X solution for the whole plate, pour all of the Enzyme Conjugate Buffer into the Enzyme Conjugate 11X vial. Mix gently.
Use within 1 day.

Number of strips	Enzyme Conjugate 11X	Enzyme Conjugate Buffer
12 strips	1 vial	1 vial
8 strips	0.36 mL	3.6 mL
4 strips	0.18 mL	1.8 mL

Wash Buffer:
Dilute 21X stock with distilled water to make 1X solution.

Note:
Glucagon ELISA #10-1281-01, Cite this, **(Mercodia Cat# 10-1281-01, RRID:AB_2783839)**

Troubleshooting

1 Prepare enzyme conjugate 1X solution and wash buffer 1X solution.

2 Prepare sufficient microplate wells to accommodate Calibrators, controls and samples in duplicate.

3 Pipette 10 µL each of Calibrators, controls and samples into appropriate wells.

4 Add 50 µL enzyme conjugate 1X solution to each well and attach the plate sealer.

5 Incubate on a plate shaker (700-900 rpm) over night (18-22h) at 2–8ºC

6 Wash 6 times with 700 µL wash buffer 1X solution per well using an automatic plate washer with overflow-wash function. After final wash, invert and tap the plate firmly against absorbent paper.
Do not include soak step in washing procedure.Or manually, Discard the reaction volume by inverting the microplate over a sink. Add 350 µL wash solution to each well. Discard the wash buffer 1X solution, tap firmly several times against absorbent paper to remove excess liquid.
Repeat 5 times. Avoid prolonged soaking during washing procedure.

7 Add 200 µL Substrate TMB.

8 Incubate on the bench for 30 minutes at room temperature (18–25ºC).

9 Add 50 µL Stop Solution to each well. Place plate on a shaker for approximately 5 seconds to ensure mixing.

10 Read optical density at 450 nm and calculate results. Read within 30 minutes.