Aug 10, 2023

Measurement of GLP-1 release in cell supernatant from Hutu-80 enteroendocrine cells via ELISA

DOI
https://dx.doi.org/10.17504/protocols.io.j8nlkokm6v5r/v1

Roxana Mezabrovschi[1], Rachel Bates[1]

[1]University College London, Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD, 20815.

Sofia Koletsi
University College London, University of London

DOI: https://dx.doi.org/10.17504/protocols.io.j8nlkokm6v5r/v1

Protocol Citation: Roxana Mezabrovschi, Rachel Bates 2023. Measurement of GLP-1 release in cell supernatant from Hutu-80 enteroendocrine cells via ELISA . **protocols.io** https://dx.doi.org/10.17504/protocols.io.j8nlkokm6v5r/v1

Protocol status: Working
We use this protocol and it's working

Created: July 26, 2023

Last Modified: May 31, 2024

Protocol Integer ID: 85525

Keywords: ASAPCRN, cell supernatant, elisa kit, assay, glucagon, elisa

Funders Acknowledgements:
Aligning Science Across Parkinson's
Grant ID: ASAP-000420

Disclaimer

This protocol was adapted from Cat. # EGLP-35K to optimise the seeding density of the assay based on the cell line used (Hutu 80 enteroendocrine cells RRID: CVCL_1301), moreover, cell line used and media conditions used to characterise GLP-1release.

The lowest level of GLP-1 that can be detected by this assay is 2 pM (100ul plasma sample size).

Abstract

This protocol describes the method of use for the Glucagon-Like Peptide-1 (Active) ELISA Kit 96-Well Plate (Cat. # EGLP-35K) to measure GLP-1 release (pM) from supernatant. The assay should be run in duplicate.

Materials

GLP-1 (Active) ELISA Plate
Coated with anti-GLP-1 Monoclonal Antibody Quantity: 1 plate
Preparation: Ready to use

Adhesive Plate Sealer
Quantity: 1 Sheet
Preparation: Ready to use

10X Wash Buffer Concentrate
10X concentrate of 10 mM PBS Buffer containing Tween 20 and Sodium Azide. Quantity: 50 mL
Preparation: Dilute 1:10 with deionized water

GLP-1 (7-36) amide ELISA Standards
GLP-1 (7-36 amide) in Assay Buffer: 2, 5, 10, 20, 50 and 100 pM Quantity: 1 mL/vial
Preparation: Ready to use

ELISA GLP-1 (Active) Quality Controls 1 and 2
Various peptides including GLP-1 (7-36 amide) in QC Buffer. Quantity: 1 mL/vial
Preparation: Ready to use

GLP-1 (Active) Assay Buffer
0.05M PBS, pH 6.8, containing proprietary protease inhibitors, with Tween 20, 0.08% Sodium Azide and 1% BSA.
Quantity: 25 mL
Preparation: Ready to use

GLP-1 (Active) Detection Conjugate
Anti GLP-1-Alkaline Phosphate Conjugate. Quantity: 21 mL
Preparation: Ready to use

Substrate (Light sensitive, avoid unnecessary exposure to light)
Quantity: 10 mg
Preparation**:** Hydrate in 1 mL deionized water just before use. Use at 1:200 dilution in substrate diluent (e.g. 100 uL hydrated substrate in 20 mL substrate diluent). Dilute fresh each time just before use.

Substrate Diluent (Light sensitive, avoid unnecessary exposure to light)
Quantity: 21 mL
Preparation: Ready to use

Stop Solution
Quantity: 6 mL
Preparation: Bring to room temperature before use. Mix thoroughly to ensure no precipitate remains.

Cell Culture Medium
Specific to cell type.

Sterile 24-well plates
Quantity: 2

PBS (1X)
Preparation: Ready to use

Reagents for you experimental media conditions
Varies between experiments.

DPP-1V Inhibitor
Quantity: 10ml (Cat. DPP4-010)
Preparation: Ready to use - to be purchased separately. (Store -20 degrees).

Before start

All reagents should be warmed to room temperature before proceeding.

Day 1

1 Plate cells in sterile 24-well plates at 1.5×10^5 per well and leave Overnight in a 37 °C incubator with stable CO2 conditions overnight.

Day 2

5m

2 Check cells are healthy under a microscope.

3 In a laminar flow hood aspirate medium and wash with PBS 3 times, adding 500 µL each time.

4 Once the final PBS wash is complete add in 500 µL of the test media made up for your desired experiment (e.g., high glucose media or 2-Deoxy-D-glucose) and place back in the incubator for 02:00:00 . 2h

5 Make up the **Wash Buffer** using the **10X Wash Buffer Concentrate** and dilute 1:10 with deionised water.

6 Once the plate has finished incubating, move over the 500 µL supernatant into a new sterile 24-well plate and add DPP-IV inhibitor immediately (1:100).to be carried out in a laminar flood hood. The cells may be stored at -80 °C for further analysis at a later date.

7 In each well of the ELISA plate, add 300 µL of diluted **Wash Buffer** (or in two intervals of 150 µL). Incubate at Room temperature for 00:05:00 . Decant excess buffer and blot with absorbent towels. 5m

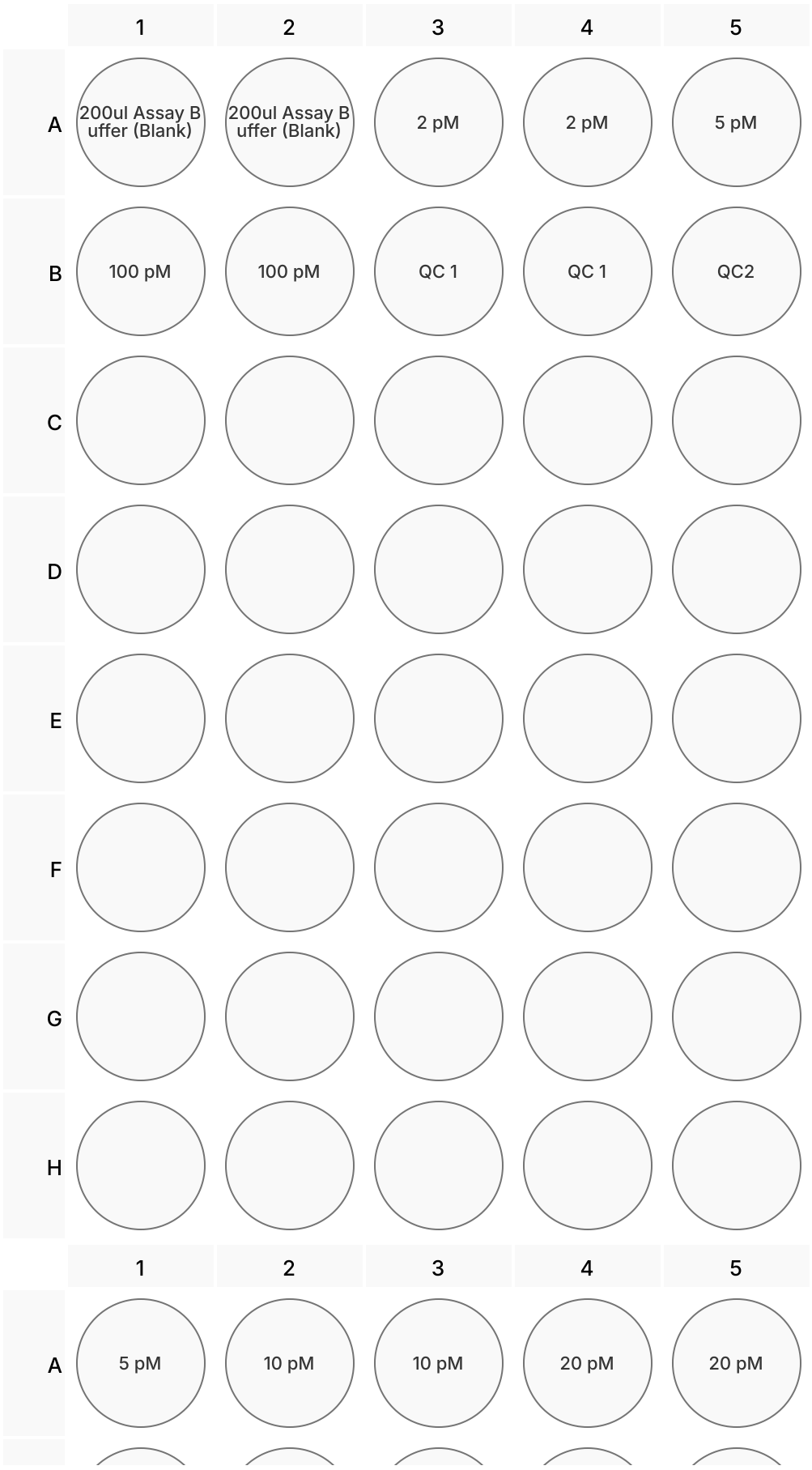
8 Add 200 µL **Assay Buffer** to NSB (non-specific binding) wells A1, A2.

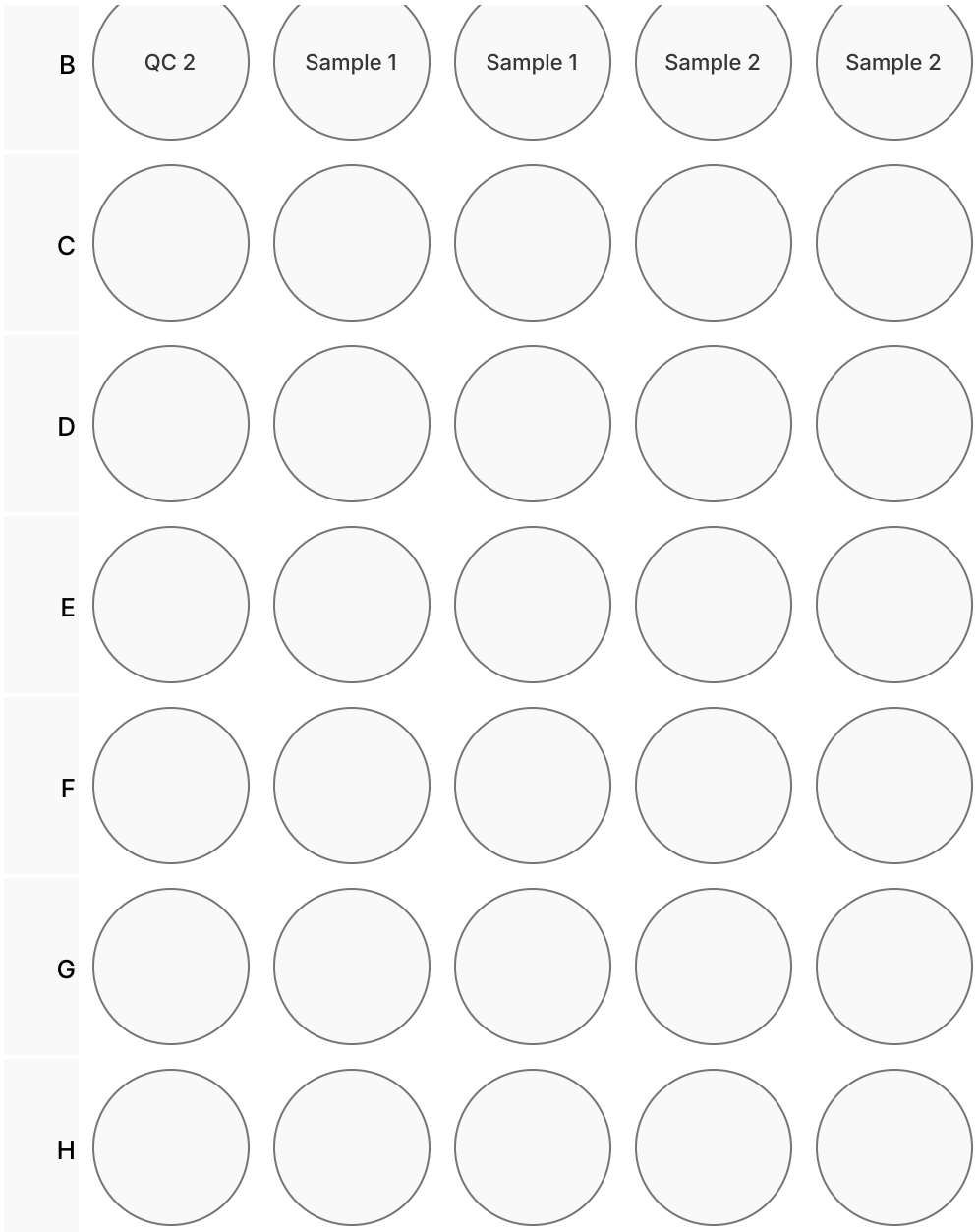
9 Add 100 µL **Assay Buffer** to the remaining wells you wish to load your samples in.

10 Add 100 µL standards in ascending order to wells - standards come preprepared.

11 Then load 100 µL of QC1 and QC2 to the plate in seperate wells.

12 Add 100 µL of your samples from the 24-well plate in the remaining wells (already containing 100 µL of the Assay Buffer). Plate layout should resemble the below.


1
2
3
4
5
A
200ul Assay B uffer (Blank)
200ul Assay B uffer (Blank)
2 pM
2 pM
5 pM
B
100 pM
100 pM
QC 1
QC 1
QC2
C
D
E
F
G
H
1
2
3
4
5
A
5 pM
10 pM
10 pM
20 pM
20 pM


B
QC 2
Sample 1
Sample 1
Sample 2
Sample 2
C
D
E
F
G
H
1
2
A
50 pM
50 pM
B
etc...
C

13 For good mixing, lightly agitate the plate.

14 Cover the plate with plate sealer. Incubate Overnight (20 to 24 hours) at 4 °C .

Day 3

2h 35m

15 *All reagents should be warmed to* Room temperature *before proceeding.*

Decant liquid from plate and tap out excess fluid on absorbent towels.

16 Wash the plate 5 times with 300 µL pre-diluted **Wash Buffer** per well with 00:05:00 incubation at Room temperature in **Wash Buffer** with the fourth wash. Tap out excess buffer on absorbent towels after the fifth wash.

5m

17 Immediately add 200 µL **Detection Conjugate** (is ready to use) in each well. Incubate 02:00:00 at Room temperature then decant.

2h

18 Whilst the plate is incubating dilute the **Substrate.** .

18.1 Hydrate in 1 mL deionized water just before use.

18.2 Use at 1:200 dilution in **Substrate Diluent** (e.g. 100 µL hydrated substrate in 20 mL substrate diluent). Dilute fresh each time just before use.

19 Wash the wells 3 times with 300 µL diluted **Wash Buffer**. Tap out excess buffer on absorbent towels.

20 Add 200 µL diluted **Substrate** in each well.

21 Measure fluorescence on a plate reader at an excitation/emission wavelength of 360/460 every 00:05:00 for a minimum of 00:20:00 . 25m

22 If sufficient fluorochrome has been generated, add 50 µL **Stop Solution** (mix thoroughly to ensure no precipitate remains) to each well in the same order as the **Substrate** was added. Incubate 00:05:00 at Room temperature in the dark to arrest phosphatase activity. 5m

23 Read plate on a fluorescence plate reader with an excitation/emission wavelength of 360/460.

Protocol references

Nathan DM, Schreiber E, Fogel H, Mojsov S, Hebener JF. Insulinotropic Action of Glucagon-like peptide-1 (7-37) in Diabetic and Nondiabetic Subjects. Diabetes Care 15: 270-276, 1992
Kieffer TJ, McIntosh CHS, Pederson RA. Degradation of Glucose-Dependent Insulinotropic Polypeptide (GIP) and Truncated Glucagon-Like Peptide (GLP-1) in vitro and in vivo by Dipeptidyl Peptidase IV. Endocrinology 136: 3585-3596, 1995
Tijsen P. “Practice and Theory of Enzyme Immunoassays” in Burdon RH and Knippenberg PH (Ed.), Laboratory Techniques in Biochemisrty and Molecular Biology. Amsterdam/NY: Elsevier, 1985
Christopoulos TK and Diamandis EP. “Fluorescence Immunoassays” in Diamandis EP and Christopoulos TK (Ed.), Immunoassay. Academic Press, 1996
Holst JJ, Cathrin Orskov, Bolette Hartmann, Carolyn F. Deacon : Postranslational processing of proglucagon and postsecretory fate of proglucagon products; in Fehmann HC, Goke B (eds) : The Insulinotropic Gut Hormone Glucagon-Like Peptide-1. Front Diabetes. Basel, Karger, 1997, vol 13, pp 24-48