# One-step Fast Human Glucagon ELISA

For the quantitative determination of human Glucagon concentrations in cell culture supernates, serum, and plasma.

## INTRODUCTION

Glucagon is a 29 amino acid polypeptide hormone, produced in the pancreas. It plays a vital role in glycogenolysis, lipolysis, gluconeogenesis, and ketogenesis. The Glucagon precursor mRNA is expressed by alpha cells of the pancreas, L cells of the intestine, and in the brain, but only the pancreatic alpha -cells are able to produce Glucagon. In normal metabolism, Glucagon normally affects on responding to low blood glucose (hypoglycemia) and is downregulated in response to high blood glucose (hyperglycemia).

Tribioscience's Fast Human Glucagon ELISA is a solid phase ELISA designed to measure glucagon levels in cell culture supernatants. serum, and plasma. The main feature is that the kit uses our novel proprietary approaches to make easy combinations of samples and detection into a one-step instead of the complicated traditional methods. It makes the assay simple, easy, accurate, and ultra-sensitive. The measurement can detect the concentration as low as 10 pg/mL, and finished in 2 hours, not need 5-6 hours (Fig. 1). The detection range is from 10 to 2000 pg/mL. The levels of human glucagon samples are parallel to the standard curves obtained using the kit standards linearly. Therefore, the kit can be used to determine relative mass values for natural human glucagon.

## PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique (See Fig. 1). A monoclonal antibody specific for human glucagon was pre-coated onto a microplate. Standards or samples,and HRP-conjugated glucagon antibody are pipetted into the wells, and incubated together. Following a wash to remove any unbound antibody and samples, an **ultra-sensitive TMB substrate solution** is added to the wells for color develops. The color intensity is in proportion to the amount of bound in the initial step. The intensity of the color is measured by plate reader at 450nm.

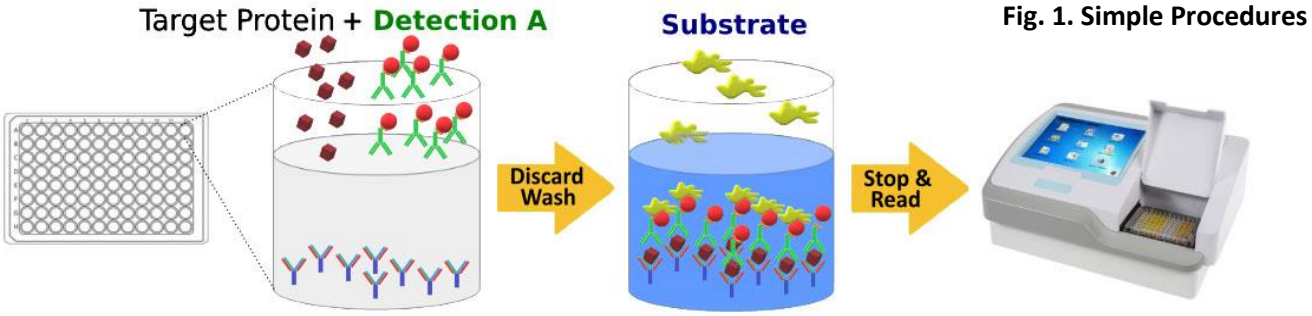



**Fig. 1. Simple Procedures**

## KIT CONTENT AND STORAGE CONDITIONS

| PART | PART# | DESCRIPTION | STORAGE OF OPENED/ RECONSTITUTED |
|---|---|---|---|
| Human Glucagon Microplate | TBS3244A | 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human glucagon. | Return unused wells to the foil pouch. Reseal along the entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C. |
| Human Glucagon Standard | TBS3244B | 50 µl of Recombinant human glucagon (50ng/mL). | Aliquot and store at -20 °C for up to 1 month in a manual defrost the freezer. Avoid repeated freeze-thaw cycles. |
| Detection A | TBS3244C | 2.1 ml of HRP- human glucagon antibody. | May be stored for up to 3 months at 2-8 °C. |
| Assay Diluent | TBS3244D | 12 ml of a buffered protein base with preservatives. | |
| Wash Buffer | TBS3000W | 12 ml of concentrated solution (10x). | |
| TMB Substrate | TBS3000T | 12 ml of ultra-sensitive TMB substrate. | |
| Stop Solution | TBS3000S | 6 ml of 2 N sulfuric acid. | |

Store the unopened kit at 2-8 ℃. Do not use past kit expiration date.
The kit contains sufficient materials to run an ELISA on one 96 well plate.

## PRECAUTIONS

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

## REAGENT PREPARATION

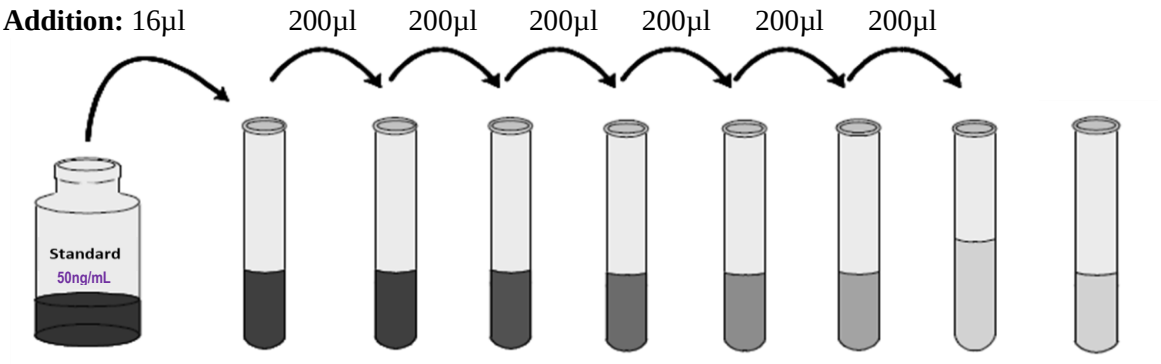
**Bring all reagents to room temperature before use.**

**Wash Buffer:** Add 12 mL of Wash Buffer Concentrate (10x) to 108 mL of deionized distilled water to prepare 120 mL of Wash Buffer (*If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved).*

**Human Glucagon Standard Preparation:** Label test tubes as #1 through #8. Pipet 384 µL of 1x Assay Diluent into tube #1, and 200 µL into tubes #2 to #8 **as diagram below.**

1. Add 16 µL of the Human Glucagon Standard stock solution (50ng/mL) to tube #1 and mix.
2. Make 2x serial dilutions of the standard using the Tube#1(2000pg/mL standard solution) from Tube #2 through #7 with sequential transfer of 200 µL to the next concentration. Mix each tube thoroughly before the next transfer. The standard concentration in tube 1 through 7 will be 2000, 1000, 500, 250, 125, 62.5, and 31.25pg/mL. Tube# 8 is Standard 0.

**Fig.2 Diagram for Human glucagon standard preparation**




| | Std1 | Std2 | Std3 | Std4 | Std5 | Std6 | Std7 | Std8 |
|---|---|---|---|---|---|---|---|---|
| **Assay Buffer (µL)** | 384 | 200 | 200 | 200 | 200 | 200 | 200 | 200 |
| **Addition** | Stock | Std1 | Std2 | Std3 | Std4 | Std5 | Std6 | |
| **Addition Vol. (µL)** | 16 | 200 | 200 | 200 | 200 | 200 | 200 | 0 |
| **Final Conc (pg/ml)** | 2000 | 1000 | 500 | 250 | 125 | 62.5 | 31.25 | 0 |

## ASSAY PROCEDURE

**Bring all reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples be assayed in duplicate.**

1. Add 80 µL of standard, sample, or control per well.
2. Add 20 µL of **Detection A** to the above standard and sample of each well, thoroughly mix. Cover with the adhesive sealer. Incubate at **RT for 2 hours**.
3. Aspirate each well, and wash for 3 times by filling each well with 300 µL Wash Buffer (*Complete removal of liquid at each step is essential to good performance*). After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
4. Add 100 µL of **TMB Substrate** to each well. Incubate **at RT for 10-20min** (*Protect from light*). The color becomes blue.
5. Add 50 µL of **Stop Solution** to each well. The color in the well should change from blue to yellow (gently tap the plate to ensure thorough mixing).
6. Determine the optical density of each well within 20 minutes, using a microplate reader at 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

## CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample subtract the average zero standard optical density (O.D.).

Create a standard curve using computer software capable of generating a four-parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the Y-axis against the concentration on the X-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

## TYPICAL DATA

This standard curve($R^2$=1.00) is provided for demonstration only. A standard curve should be generated for each set of samples assayed. Fig. 3 is an example of typical Data.

## SENSITIVITY

The minimum detectable dose (MOD) of human is typically 10pg/ml.
The Intra-assay CV is 4.56% the Inter-assay CV is <10%.

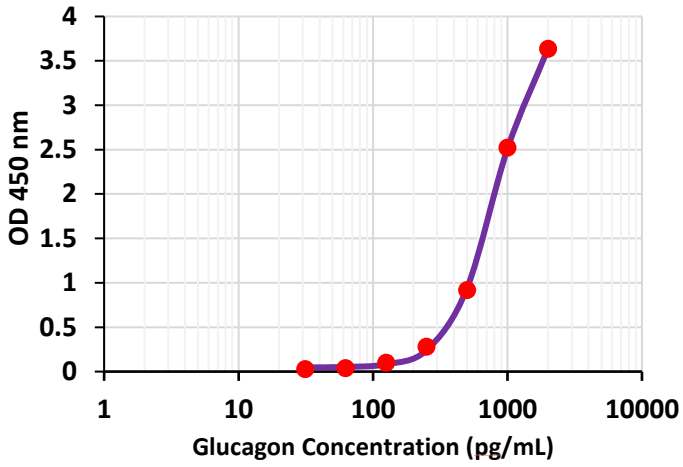
## SPECIFICITY

This assay recognizes natural and recombinant human glucagon.
No cross-reactivity with others.

## RELATIVE PRODUCTS

Human IL-1ß ELISA (TBS3219)
Human IL-2 ELISA (TBS3220)
Human IL-4 ELISA (TBS3221)
Human IL-6 ELISA (TBS3223)
Human IL-7 ELISA (TBS3224)
Human IL-8 ELISA (TBS3225)
Human IL-10 ELISA (TBS3226)
Human IL-13 ELISA (TBS3227)
Human IL-17 ELISA (TBS3228)
Human IL-22 ELISA (TBS3229)
Human Insulin ELISA (TBS3236)
Human IL-33 ELISA (TBS4245)
Human VASN ELISA (TBS4246)
Human IFN-gamma ELISA (TBS3230)
Human TGF- ß1 ELISA (TBS3232)
Human GM-CSF ELISA (TBS3233)
Human MIP-1α ELISA (TBS3234)
Protein Cell Lysis Buffer (TBS5001)
Protein Assay Kit (TBS2005)
TMB Substrate System (TBS5021)

**Fig.3 Glucagon Standard Curve**

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