Version 25-11/24

**User's Manual**

# Tirzepatide ELISA Kit

REF **DEIA-JY2495**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

**Creative Diagnostics**

**Address: 45-1 Ramsey Road, Shirley, NY 11967, USA**

**Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe)** **Fax: 1-631-938-8221**

**Email: info@creative-diagnostics.com** **Web: www.creative-diagnostics.com**

# PRODUCT INFORMATION

## Intended Use

Tirzepatide is a dual active agonist of both Glucagon-like Peptide-1 (GLP-1) and Glucosedependent Insulinotropic Polypeptide (GIP) receptors. It acts as an incretin hormone with important effects on glycemic control and body weight regulation, particularly relevant in people with Type-II Diabetes (T2D). Tirzepatide is used as active component of Mounjaro®.

This ELISA was developed with serum from rabbits immunized with Tirzepatide coupled to a carrier protein.

## Principles of Testing

This ELISA kit is a competitive immunoassay. The antiserum is captured by antibodies coated on a 96-well plate. A constant concentration of Bt-tracer (biotinylated tracer) and varying concentrations of unlabeled standard or sample peptide compete for binding specifically to the antiserum. Captured Bt-tracer is subsequently bound by streptavidin-conjugated horseradish peroxidase (SA-HRP), which produces a soluble colored product after a substrate is added.

The standard is used to make a standard curve in the range specified in the kit's datasheet. Standard curves are S-shaped (on a semi-log plot) but for a few kits they appear to be almost linear over the kit's range. The measuring range is the range of standard concentrations near the middle or near the IC050 of the standard curve. Unknown sample concentrations are measured by comparing their absorbance with the standard curve.

We include sufficient reagents for 96 determinations.

Immunogen: Synthetic peptide H-Tyr-{Aib}-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Tyr-Ser-Ile-{Aib}-Leu-Asp-Lys-Ile-Ala-Gln-{diacid-gamma-Glu-(AEEA)2-Lys}-AlaPhe-Val-Gln-Trp-Leu-Ile-Ala-Gly-Gly-Pro-Ser-Ser-Gly-Ala-Pro-Pro-ProSer-NH2 coupled to carrier protein.

## Reagents And Materials Provided

1. ELISA Buffer Concentrate (50 ml 20x concentrate)
2. 96-well immunoplate with plate sealer
3. Antiserum (lyophilized powder)
4. Biotinylated tracer (lyophilized powder)
5. Streptavidin-HRP (100 µl 200x concentrate)
6. TMB substrate solution (1.5 ml)
7. TMB substrate buffer (15 ml citrate buffer)
8. Stop Solution 2 N HCl (15 ml)
9. Kit Datasheet/Protocol Insert

## Materials Required But Not Supplied

1. Standard Peptide. Purchase synthetic peptide with sequence identical to the antigen equence listed on the

datasheet.

2. 96-well microtiter plate reader set up to measure 450 nm and 650 nm.
3. 96-well plate washer and shaker (optional)
4. Distilled, deionized or USP water
5. Curve fitting software (optional, use free online services or statistics software packages)
6. Test tubes, pipettes and various other standard laboratory items
7. Extraction Kit (with 50 columns, Buffers A and B)

   Buffer A

   Buffer B

   SEP columns (200 mg)

   SEP column adapters

## Storage

After you receive the kit, store it in the refrigerator (4 - 8°C) for up to 1 year. Long term storage, improper storage conditions and large temperature fluctuation cycles may cause precipitates in the TMB solution and in the ELISA buffer concentrate. These precipitates should not affect the assay noticeably. Nevertheless, if you observe such precipitates, we recommend removing them by filtration prior to usage

## Specimen Collection And Preparation

### SUGGESTED PROTOCOL FOR SAMPLE EXTRACTION

You can use standard to determine if extraction is required. For example, if you are working with serum, you may spike it with known amounts of standard and check if they are accurately determined by the assay with and without extraction. Extraction eliminates potentially interfering substances, such as albumin.

Extraction may also be necessary to concentrate the sample to within the measuring range. As with any purification technique, recovery of the desired substance is likely to be incomplete. Therefore, both optimization and quantification of the extraction procedure are recommended for more accurate determinations. While we cannot provide you with extraction optimization and quantification protocols, we have included enough standard in the kit should you wish to use it for this purpose

### C18 Sep-Column Extraction Method

The following generic protocol is meant to help users with extracting their samples. It should be applicable to different biological fluids but should not be thought of as an optimized protocol for any particular antigen.

**Required Materials (must be purchased seperately)**

SEP-Column containing 200 mg of C18

Buffer A: 1% trifluoroacetic acid (TFA, HPLC Grade). (Acidifies plasma sample to remove interfering proteins such as albumin)

Buffer B: 60% acetonitrile (HPLC Grade), 1% TFA, and 39% distilled water. (Elutes peptide from column) You may also consider purchasing Extraction kits (Contact CD for Cat. No.), which include SEPcolumns and buffers A & B for 50 samples.

**Withdrawal and Preparation of Plasma**

Collect blood samples (2 - 6 ml) into a chilled syringe and transfer into a polypropylene tube containing EDTA (1 mg/ml of blood) as an anticoagulant and Aprotinin (500 kIU/ml of blood) as a protease inhibitor at 4°C. Do not use heparinized tubes as they may interfere with the assay. Vacutainers with EDTA are acceptable. Centrifuge blood at 1,600 x g for 15 minutes at 4°C. Collect the top (plasma) layer. Proceed to extraction immediately or freeze at -70°C for later use.

**Extraction Procedure**

1. Add an equal amount of Buffer A to the plasma.
2. Centrifuge at 6,000 x g to 17,000 x g for 20 minutes at 4°C.
3. Transfer supernatant to a new tube discarding any pellet that may be present.
4. Equilibrate a SEP-Column by washing with 1 ml Buffer B followed by 3 x 3 ml Buffer A.
5. Load the plasma solution onto the equilibrated SEP-Column.
6. Slowly wash the column with Buffer A (3 ml, twice) and discard the wash. A light vacuum (10 sec/drop) may be applied to the column.
7. Elute the peptide slowly with Buffer B (3 ml, once) and collect eluant in a polypropylene tube. A light vacuum may be applied as in previous step.
8. Freeze-dry eluant to dryness using a dry ice/methanol bath to freeze the sample and a centrifugal concentrator to evaporate it.
9. Dissolve the residue in a suitable volume of ELISA buffer such that the concentration of the substance of interest will fall close to the IC50 (within the measuring range).

## Reagent Preparation

1. **Sample extraction.** Sample extraction is recommended for serum or plasma samples used in EIAH - high sensitivity absorbance assays. It may be less important for tissue culture samples. See "Suggested Protocol for Sample Extraction" for details. The kit may still be used without extraction but this may cause unexpected results due to the possible binding between serum proteins and kit components.
2. **Sample concentration.** The concentration of the target molecule must be within the measuring range of the kit (most precise results will be achieved in the linear part of the standard curve around the IC50). If the concentration range of your sample is difficult to estimate, prepare it at different concentrations such that one of the samples should lie within the measuring range.
3. **Standard.** Purchase synthetic peptide corresponding to the antigen sequence. Make a stock solution from which to prepare the serial dilutions for the standard curve. Make serial dilutions of the purchased standard to cover the range of the kit, refere to datasheet for standard range and suggested dilutions.

**PREPARE KIT COMPONENTS**

Lyophilized kit components should not be re-hydrated until they are needed. Please read the complete protocol before proceeding.

1. **Equilibrate unopened kit components to room temperature.** Avoid accumulation of moisture, do not open reagents and immunoplate while they are cold.
2. **ELISA buffer.** Dilute the ELISA buffer concentrate 1 in 20 with water and mix well. Example: mix the 50 ml contained in the kit with 950 ml of water.

3. **Standard stock.** Prepare a 10 µg/ml standard stock solution in ELISA buffer.
4. **Standard curve.** Make serial dilutions of the standard to cover the range of this kit. Please check the included datasheet for the appropriate range and dilution suggestions.
5. **Antiserum.** Add 5 ml of ELISA buffer and mix gently.
6. **Biotinylated tracer.** Add 5 ml of ELISA buffer to the vial of lyophilized biotinylated peptide and mix gently. Please check the datasheet for exceptions.

## Assay Procedure

1. **ELISA buffer** Standard dilutions, samples, antiserum and Bt-tracer are reconstituted and used in ELISA buffer. If there is no interference with the kit's components, you should use your own diluent for your samples and standards. However, the standard curve should show similar characteristics as the one from the datasheet.
2. **Room Temperature.** Reagents, samples, and the plate should be brought to room temperature before use.
3. **Shakers.** Shakers (optional) may help lower the experimental variation of duplicates (recommended at 60 rpm).
4. **Blank Wells.** Blanks will give you the background to be subtracted from all readings. These should not be confused with the "S0 Standards" which contain no standard peptide and which will yield the highest readings. Blank readings will not influence concentration calculations - thus, they are optional.

**LAYOUT**

Seven-Point Standard Curve Layout

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | Blk | Blk | U1 | U1 | U9 | U9 | U17 | U17 | U25 | U25 | U33 | U33 |
| B | S1 | S1 | U2 | U2 | U10 | U10 | U18 | U18 | U26 | U26 | U34 | U34 |
| C | S2 | S2 | U3 | U3 | U11 | U11 | U19 | U19 | U27 | U27 | U35 | U35 |
| D | S3 | S3 | U4 | U4 | U12 | U12 | U20 | U20 | U28 | U28 | U36 | U36 |
| E | S4 | S4 | U5 | U5 | U13 | U13 | U21 | U21 | U29 | U29 | U37 | U37 |
| F | S5 | S5 | U6 | U6 | U14 | U14 | U22 | U22 | U30 | U30 | U38 | U38 |
| G | S6 | S6 | U7 | U7 | U15 | U15 | U23 | U23 | U31 | U31 | U39 | U39 |
| H | S0 | S0 | U8 | U8 | U16 | U16 | U24 | U24 | U32 | U32 | U40 | U40 |

Blk = blank S = standards U = unknown samples

1. **Into each well of the immunoplate add**

**50 µl standard or** (in ELISA buffer)

**25 µl antiserum** (in ELISA buffer)

Add 75 µl ELISA buffer to blank wells.

2. **Incubate at room temperature for 1 hour.**
3. **Add 25 µl Bt-tracer** (in ELISA buffer) per well including the blanks.
4. **Incubate at room temperature for 2 hours**. Shorter incubation times may result in low signal.
5. **Wash immunoplate 5 times with 300 µl per well of ELISA buffer**. Be careful not to cross-contaminate between wells in the first wash/dispensing cycle. In each wash cycle empty plate contents with a rapid

flicking motion of the wrist, then gently blot dry the top of plate on paper towels. Dispense 300 µl of ELISA buffer into each well and gently shake for at least a few seconds. Thorough washing is essential.

6. **Add 100 µl per well of streptavidin-HRP.** Tap or centrifuge the SA-HRP vial to collect all liquid contents on the bottom of the vial. Dilute 1/200 in ELISA buffer (60 µl in 12 ml) and mix gently. Add 100 µl to all wells, including the blanks.
7. **Incubate at room temperature for 1 hour.**
8. **Prepare TMB chromogenic solution** immediately before use by mixing 20 parts of the Substrate buffer (citrate, brought to room temperature) with 1 part TMB - H2O2 Stock Solution. This dilution should be used within 15 minutes after preparation.
9. **Wash immunoplate 5 times (see step 3)**.
10. **Add 100 µl per well of freshly prepared TMB chromogenic solution**. Add to all wells, including the blanks.
11. **Incubate at room temperature (usually 10 minutes)**. This can be adapted according to how fast the color reaction takes place. You may read the developing blue color at 650 nm to decide when to stop the color reaction.
12. **Terminate reactions by adding 100 µl 2 N HCl per well.**
13. **Read absorbance at 450 nm within 15 minutes** (and optionally at 650 nm for background correction).

## Quality Control

The kit's IC50, or the shape of the standard curve, may exhibit some variation but this will not affect the kit's accuracy in the measuring range. The kit accurately measures sample peptides if the following conditions are met:

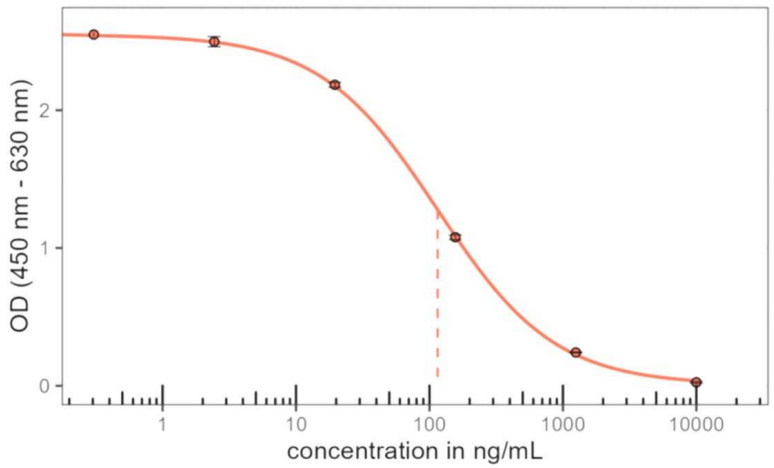
1. **Both samples and standards must be measured in the same diluent and under the same conditions** (same microtiter plate).
2. **The kit's antiserum must not cross-react appreciably with other factors present in the sample.** Cross-reactivity tables are included with each kit. The user may wish to test the cross-reactivity with other peptides.
3. **The sample peptides must be identical to the kit's standard.** Ideally the kit's synthetic standard mimics the natural peptide perfectly. Sometimes, however, natural peptides exist as families of species related by a common or similar sequence. Also, natural peptides may be modified enzymatically or spontaneously, may exist in complexes, and may assume alternative structural forms. In these cases the kit might not measure the exact concentration of a particular natural peptide species, but it may still be used for relative average measurements.
4. **Sample extraction.** Factors present in serum can bind to EIAH kit components. The effects can vary from negligible to complete obliteration of signal. Therefore, sample extraction may be required prior to using the kit.

## Calculation

Plot data and calculate results. We recommend that you use curve fitting software for your data analysis. Plate readers often include such software packages, otherwise use free online tools that support 4 parameter logistic fitting (4PL) also called dose-response curve, i.e. https://mycurvefit.com/. Statistical software such as

Origin™, Prism™ or R are also capable of such calculations. This is, however, not essential and you may opt to plot manually on semi-log paper or use a spreadsheet program.

## Typical Standard Curve




| Suggested Preparation of Standards | | | |
|---|---|---|---|
| | ng/ml | Range: 0.31 - 10'000 ng/ml | |
| **Stock** | 10'000.0 | | |
| **S1** | 10'000.0 | 1000 µl Stock | |
| **S2** | 1250.0 | Add 125 µl S1 | + 875 µl diluent |
| **S3** | 156.25 | Add 125 µl S2 | + 875 µl diluent |
| **S4** | 19.53 | Add 125 µl S3 | + 875 µl diluent |
| **S5** | 2.44 | Add 125 µl S4 | + 875 µl diluent |
| **S6** | 0.31 | Add 125 µl S5 | + 875 µl diluent |
| **S0** | 0.00 | | 1000 µl diluent |

## Performance Characteristics

Lower LOD: 2.9 ng/ml

Average IC50: 115 ng/ml

## Detection Range

15 - 1000 ng/ml

## Specificity

Tirzepatide 100%

Semaglutide 0%

## Precautions

The physical and chemical properties of the reagents contained in this kit have been tested individually. Reagents do not contain ingredients which have been determined to be health hazards and which comprise greater than 1% of the mixture or which could be released from the mixture in concentrations that would exceed OSHA permissible exposure limits.

**Hazardous Ingredients:**

The lyophilized standard, antiserum and biotinylated tracer contain thimerosal or ProClin™ 150 as preservative.

**Physical and Chemical Data**:

Components are stable in closed containers under normal temperatures and pressures. No hazardous polymerization is known.

**Fire and Explosion Data:**

Components are non-combustible with negligible fire hazard when exposed to heat or flame. Fire fighting media should be appropriate to burning material.

**Health Hazards:**

Components may be harmful by inhalation, ingestion, or skin absorption and may cause skin irritation or eye irritation. In case of eye contact, flush eye with water and contact a physician. In case of skin contact, wash skin with soap and water.

**Reactivity Data:**

Components are stable in closed containers under normal temperatures and pressures.

**Spill and Disposal Procedures:**

For spills, ventilate area and wash spill site. For disposal, please dispose in accordance with local regulations.

**Handling and Storage Information:**

Safety glasses, gloves, and a full-length lab coat should be worn to prevent unnecessary contact.

The above information is believed to be correct but does not purport to be all-inclusive and shall be used only as a guide. It is the user's responsibility to determine the suitability of this information for the adoption of safety precautions as may be necessary.