Development and Validation of an ELISA kit for quantification of novel bispecific GLP-1/GIP drug Tirzepatide

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ABSTRACT

Background:
Tirzepatide, a dual GIPR/GLP-1R agonist, has rapidly gained prominence as a next-generation therapeutic for type 2 diabetes and obesity, with significant clinical success observed in multiple global trials. The advancement of biosimilar formulations and novel analogs has further increased the demand for robust analytical tools to support bioequivalence testing, pharmacokinetic profiling, and drug development workflows. Traditional quantification methods such as LC-MS/MS, while sensitive, are often resource-intensive, time-consuming, and require extensive method development and validation. Currently, no commercially available ELISA offers a standardized, ready-to-use format for the quantitative estimation of Tirzepatide in biological matrices.

Objective:
To develop a sensitive, quantitative, and ready-to-use competitive ELISA for Tirzepatide, enabling accurate measurement in human serum and plasma samples. The assay intends to eliminate the need for in-house coating, optimization, and assay assembly, thereby minimizing inter-laboratory variability and reducing human error.

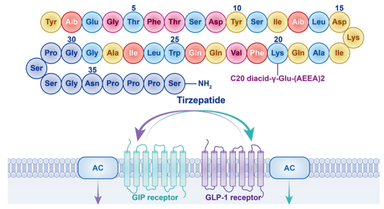
Method: The development process involved coating microplates with a recombinant human GLP-1R capture protein, followed by competitive binding with a synthetic Tirzepatide analog and detection using an HRP-conjugated anti-peptide antibody. Key parameters such as antigen coating concentration, blocking buffer, incubation conditions, and conjugate dilution were optimized to maximize signal-to-noise ratio, linearity, and assay reproducibility. Validation studies demonstrated excellent intra- and inter-assay precision, strong linearity ($R^2 > 0.99$) across a dynamic range of 31.3–4000 ng/mL, and minimal cross-reactivity with GLP-1 and other incretin peptides.

This ELISA kit offers a robust, standardized alternative to LC-MS for Tirzepatide quantification. Its implementation in drug discovery, formulation testing, and clinical research provides a scalable alternative to LC-MS workflows, and serves as a critical quality control tool for pharmaceutical development pipelines.

INTRODUCTION

Tirzepatide is a novel ***dual agonist*** of the GLP-1 and GIP receptors and has emerged as a promising ***therapeutic for type 2 diabetes and obesity*** management. With the widespread clinical adoption of branded formulations such as Mounjaro® and the advancing development of biosimilars, there is a growing demand for accurate, high-throughput tools to quantify tirzepatide in biological samples.

Tirzepatide is a synthetic 39–amino acid linear peptide engineered as a dual agonist of the glucagon-like peptide-1 receptor (GLP-1R) and glucose-dependent insulinotropic polypeptide receptor (GIPR). It exhibits high-affinity binding to both class B G-protein coupled receptors (GPCRs), leading to receptor internalization and selective activation of Gαs-mediated adenylate cyclase pathways. The resultant cAMP accumulation promotes pancreatic β-cell insulinotropic responses in a glucose-dependent manner and attenuates glucagon secretion from α-cells. Pharmacologically, tirzepatide has been ***shown to induce biased agonism***, favoring cAMP-driven signaling over β-arrestin recruitment at both receptors. This signaling profile contributes to its enhanced glycemic control, body weight reduction, and improved insulin sensitivity, as demonstrated in clinical and preclinical models.



To address the lack of available standardised quantification tools for this key new drug, we developed and validated the ***world's first commercially available ELISA kit for Tirzepatide.*** Designed as a competitive immunoassay, this kit enables sensitive, reproducible, and high-throughput quantification of tirzepatide in human serum, plasma, and cell culture supernatants.

This poster outlines the development process, assay validation, lot-to-lot reproducibility, and matrix optimization, including comparative testing against commercial drug formulations and biosimilars. Through rigorous buffer and serum spiking studies, we identified optimal assay conditions that are now integrated into the final kit, ensuring high analytical sensitivity, minimal interference, and consistent performance across biologically relevant matrices.

KIT OPTIMIZATION AND VALIDATION

ASSAY PRINCIPLE AND PROTOCOL

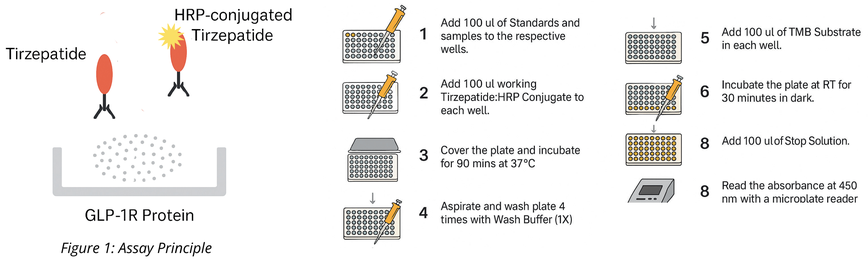


Figure 1: Assay Principle

The Tirzepatide ELISA is a competitive assay based on the binding affinity of Tirzepatide to GLP-1R protein immobilized on the microplate. Unlabeled Tirzepatide from the sample competes with a fixed concentration of HRP-conjugated Tirzepatide for binding sites. The resulting colorimetric signal, generated via TMB substrate, is inversely proportional to Tirzepatide concentration. The protocol involves co-incubation of sample and conjugate, washing, substrate development, and absorbance measurement at 450 nm.

REAGENT SPECIFICITY

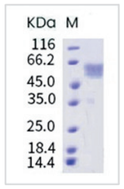


Figure 2: Gel Image of the Coat/Capture Protein

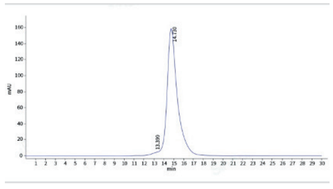


Figure 3: Chromatogram of the Coat/Capture Protein

The kit uses a recombinant GLP1r protein expressed in HEK293 as capture antigen. The protein construct is a DNA sequence encoding the human GLP1R (NP_002053.3) (Met1-Tyr145) expressed with the Fc region of human IgG1 at the C-terminus. Purity: ≥98% (HPLC)

RECOVERY AND MATRIX EFFECT

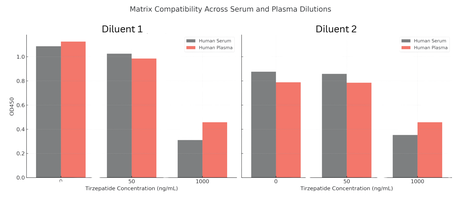


Figure 4: Serum and plasma spiking studies demonstrate consistent OD450 signals across all dilutions.

Both in-house proprietary diluents maintained matrix compatibility, with minimal interference observed at 0, 50, and 1000 ng/mL concentrations - both offering consistent and reliable performance across tested concentrations.

PRECISION AND LOT VARIABILITY

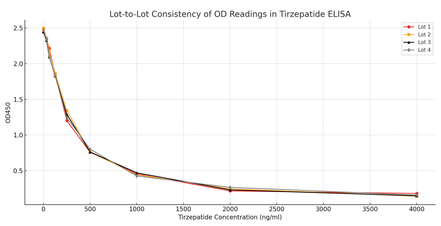


Figure 5: Overlaid OD450 curves for four independent lots showing near-identical response across the full concentration range.

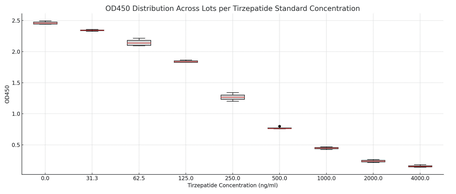


Figure 6: Distribution of OD450 values per standard across all lots, highlighting close signal spread with minimal outliers.

CROSS REACTIVITY

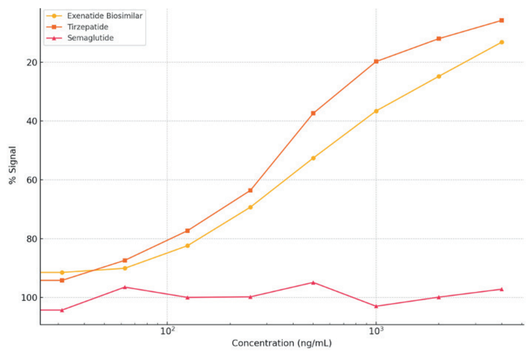


Figure 8: Tirzepatide vs Exenatide vs Semaglutide Inhibition Profile

Competitive binding results demonstrate concentration-dependent inhibition for both Tirzepatide and Exenatide, consistent with competitive assay dynamics.

- Tirzepatide exhibited a stronger inhibitory effect, reducing signal to 37.4% at 500 ng/mL and 19.8% at 1000 ng/mL, compared to Exenatide, which reduced signal to 52.6% and 36.6%, respectively.
- Semaglutide did not significantly affect assay signal across concentrations tested.

These findings confirm the higher binding affinity of Tirzepatide to GLP-1R under assay conditions, with Exenatide showing partial cross-reactivity and Semaglutide demonstrating minimal to no detectable binding.

SENSITIVITY AND CLINICAL APPLICABILITY

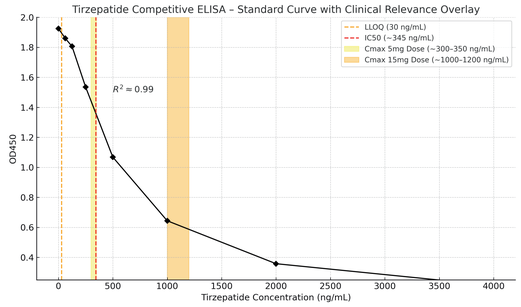


Figure 9: Standard Curve with Clinical Relevance

The Tirzepatide ELISA demonstrates an IC_{50} of ~345 ng/mL within the assay range of 0–4000 ng/mL. The assay fully encompasses clinical Cmax values observed after 5 mg (300–350 ng/mL) and 15 mg (1000–1200 ng/mL) dosing, enabling accurate quantification without modification.

PRECISION

Intra-Assay CV	<15%
Inter-Assay CV	<18%

Precision results are within acceptable limits for competitive ELISA formats, based on bioanalytical method validation guidelines.

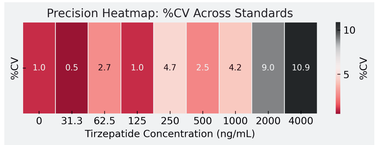


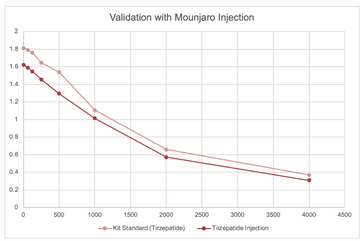
Figure 10: Average %CV across standards for n=15 assay runs

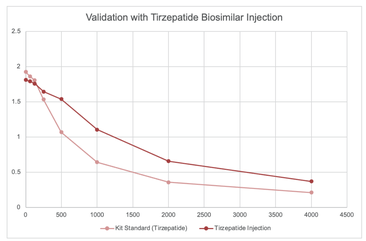
Standard Concentration (ng/ml)	Lot 1	Lot 2	Lot 3	Lot 4	% STD DEV	% CV
0	2.441	2.495	2.446	2.471	2.5	1.0
31.3	2.346	2.358	2.328	2.342	1.2	0.5
62.5	2.216	2.173	2.095	2.104	5.8	2.7
125	1.850	1.865	1.827	1.828	1.8	1.0
250	1.201	1.341	1.290	1.243	6.0	4.7
500	0.758	0.763	0.762	0.800	2.0	2.5
1000	0.455	0.443	0.470	0.426	1.9	4.2
2000	0.216	0.246	0.231	0.266	2.2	9.0
4000	0.181	0.142	0.148	0.158	1.7	10.9

Figure 7: Numerical data summary of intra-assay precision, showing %CV and % standard deviation (%STD DEV) across all concentrations.

The ELISA shows high reproducibility across four independent lots, with %CV values ≤ 5% across most of the assay range. Slightly elevated %CVs at low OD values (high analyte concentrations) are expected in competitive formats. These results confirm the assay's precision and manufacturing consistency for high-throughput and regulated use.

COMPARATIVE VALIDATION: MOUNJARO® AND TIRZEPATIDE BIOSIMILAR





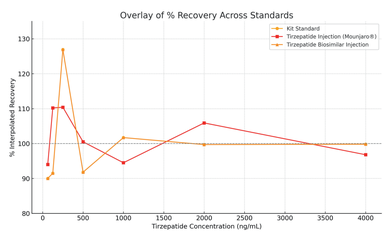


Figure 11, 12, 13: Comparative graphs for validation of the KRIBIOLISA Tirzepatide Kit with Mounjaro®, locally sourced Tirzepatide biosimilar, and the three recovery-across standards comparisions

This section demonstrates quantitative validation of the Tirzepatide ELISA kit using reference material (Mounjaro®) and a biosimilar injection. Interpolated recovery was consistent across all three sources, with most values within ±10% of ideal. At key therapeutic levels (250–1000 ng/mL), the kit standard, Mounjaro®, and the biosimilar all showed comparable binding and signal patterns, confirming assay suitability for biosimilar equivalence, comparability, and drug release studies.

Standards (ng/ml)	% Interpolated Concentration against Actual Concentration (Kit Standard)	% Interpolated Concentration against Actual Concentration (Tirzepatide Injection)	% Interpolated Concentration against Actual Concentration (Biosimilar Injection)
62.5	90	94.0	90
125	91.5	110.2	91.5
250	126.9	110.4	126.9
500	91.8	100.5	91.8
1000	101.7	94.5	101.7
2000	99.7	105.9	99.7
4000	99.8	96.8	99.8

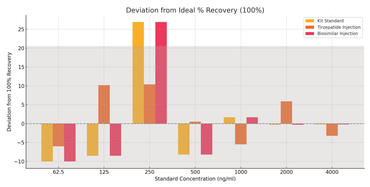


Figure 14: Interpolated concentration (% recovery). The grey box indicates ideal 100 +/- 20% accepted recovery as per US FDA Bioassay Guidelines.

CONCLUSION

The Tirzepatide Competitive ELISA developed and validated here demonstrates high sensitivity, reproducibility, and specificity for the quantification of Tirzepatide in biological matrices. Validation studies show minimal lot-to-lot variability, acceptable intra- and inter-assay precision, and consistent recovery across serum, plasma, and buffer systems.

Comparative testing against Mounjaro® and a Tirzepatide biosimilar confirms assay accuracy and clinical relevance. The finalized assay configuration, including optimized diluent and matrix handling conditions, ensures robust performance suitable for pharmacokinetic, comparability, and research applications. The assay covers the clinical Cmax concentrations of Tirzepatide following therapeutic dosing. Thus, it is suitable for pharmacokinetic evaluation and therapeutic monitoring.

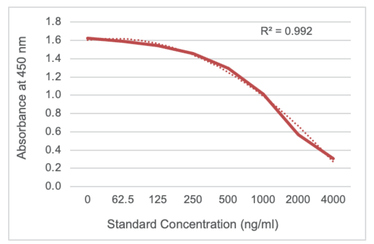


Figure 15: Typical CoA

Key Highlights

- Assay Type: Competitive ELISA
- Capture Target: GLP-1R protein
- Detection: HRP-Tirzepatide conjugate, TMB substrate, OD450 readout
- Assay Range: 30 – 4000 ng/mL
- Lower Limit of Quantification (LLOQ): 30 ng/mL
- IC_{50} Value: ~345 ng/mL

About Krishgen Biosystems:

Established 2003, Krishgen is an immunoassay manufacturer based out of Mumbai, India. Our key products include assays for mAbs, bispecific antibodies, antibody-drug conjugates, peptides as well as a wide range of cytokine and biomarker ELISA across various species.

Our products are well validated, sensitive, robust and competitively priced. As of April 2025, Krishgen ELISA have been cited in 5000+ publications worldwide. ***Learn more about Krishgen and ELISA we offer at www.krishgen.com.***