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Development of Competitive ELISA for LIRAGLUTIDE.

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INTRODUCTION

Liraglutide (NN2211) is a derivative of human Incretin (metabolic hormone) glucagon-like peptide-1(GLP-1) that is used as a long-acting glucagon-like peptide-1 receptor agonist, binding to the same receptors as does the endogenous metabolic hormone GLP-1 that stimulates insulin secretion. Marketed under the brand name **Victoza**

The product was approved for treatment of type 2 diabetes by the European Medicines Agency (EMA) on July 3, 2009, and by the U.S. Food and Drug Administration (FDA) on January 25, 2010. More recently, Liraglutide was approved by the FDA on December 23, 2014 and by the European Medicines Agency on January 23, 2015, for adults with a body mass index (BMI) of 30 or greater (obesity) or a BMI of 27 or greater (overweight) who have at least one weight-related condition.

TYPE 2 DIABETES

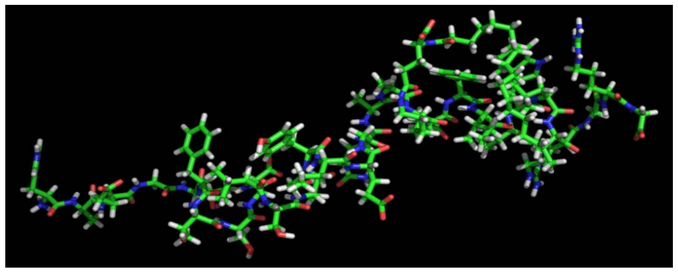
Liraglutide improves control of blood glucose. In common to various degrees with other GLP-1 receptor agonists, liraglutide has advantages over more traditional therapies for type 2 diabetes:

It acts in a glucose-dependent manner, meaning it will stimulate insulin secretion only when blood glucose levels are higher than normal, preventing "overshoot". Consequently, it shows negligible risk of hypoglycemia.

- It has the potential for inhibiting apoptosis and stimulating regeneration of beta cells (seen in animal studies).
- It decreases appetite and inhibits body weight gain, as shown in a head-to-head study versus glimepiride.
- It lowers blood triglyceride levels.

OBESITY

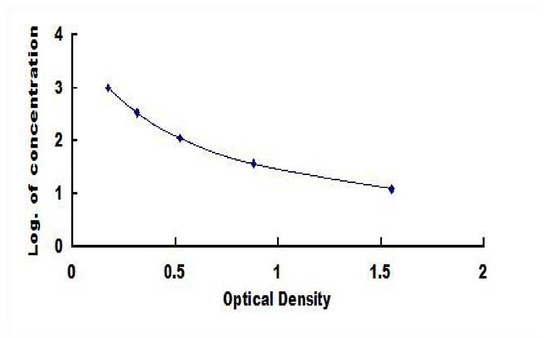
Liraglutide has been approved as an injectable adjunct to a reduced-calorie diet and increased physical activity for chronic weight management in adult patients. The specified criteria are an initial body mass index (BMI) of 30 kg/m^2 or greater (obese), or 27 kg/m^2 or greater (overweight), in the presence of at least one weight-related comorbid condition (e.g. hypertension, type 2 diabetes mellitus, or dyslipidemia).



NMR structure of Liraglutide

PRINCIPLE

The Liraglutide ELISA is a competitive immunoassay for the determination of Liraglutide. Anti- Liraglutide antibodies are coated on a 96-well plate. A constant concentration of Liraglutide-Biotin and varying concentrations of unlabeled standard or samples containing Liraglutide compete for binding to Anti- Liraglutide antibodies coated on microwell. Captured Liraglutide-Biotin is subsequently bound by Streptavidin-HRP, which produces a soluble colored product after a substrate is added. The enzyme reaction is stopped by dispensing an acidic solution (H_2SO_4) into the wells after 10-30minutes. The optical density (OD) of the solution at 450 nm is inversely proportional to the amount of bound Liraglutide molecule present in standards or samples.



Typical Standard curve for Liraglutide ELISA. Reading was taken after adding the substrate at 450nm and reaction was stopped by an acid.

SPECIFICITY

The antibodies in this kit are specific for Liraglutide peptide. The standard used is a synthetic peptide for Liraglutide.

SENSITIVITY

Limit of Detection (LOD) – 0.2ng/ml

Limit of Quantitation (LOQ) – 0.24ng/ml

ASSAY RANGE: 0.24ng/ml – 250 ng/ml

CROSS REACTIVITY:

Liraglutide: - 100%

GLP-1(7-37) -: 50% GLP-1

(7-36) amide -: 0%

Glucagon -: 0%

GLP-2 -: 0%

PRECISION

- Both samples and standards must be measured in the same diluent and under the same conditions.
- The kit's antibodies must not cross-react appreciably with other factors present in the sample. The user may wish to test the cross-reactivity with other peptides.
- CV (%) = SD/mean X 100
- Intra-Assay: CV<10%
- Inter-Assay: CV<12%

RECOVERY

Recovery rate was found to be greater than 91% with native human serum and plasma samples when spiked with liraglutide in samples.

CONCLUSION

The kit developed at Krishgen gives rapid, accurate, reproducible, results for estimation of liraglutide in human serum.