

INSULINASE INHIBITION
COCKTAIL
D05011

For research laboratory use only.
Not for human diagnostic use.



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December 2011

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INTRODUCTION

NOTES

Insulin (51 amino acids, 5808 Da) is synthesized from its precursors preproinsulin and proinsulin (hPI; 86 amino acids) in the beta cells of the pancreatic islets of Langerhans.

The main analytical pitfalls in insulin measurement in serum are related to (a) hemolysis, (b) circulating anti-insulin autoantibodies, and, for the past few years, (c) the reactivity (or lack of reactivity) of rapid- or long-acting pharmacological insulin analogs in sera (1).

Hemolyzed samples contain an insulin-degrading enzyme and should not be analyzed unless they can be handled at 4 °C within 2 to 3 h or an insulinase inhibitor cocktail has been added in the blood collection tube to prevent insulin degradation.

This inhibitor cocktail shall have no impact on the assay robustness, and therefore have been validated with the analytical method used.

This Inhibitor cocktail has been developed and validated with Bertin Pharma mouse/rat insulin EIA kit (#A05105). In case you would like to use it with a different assay, check for possible interferences.

The protocol hereafter detailed the sample and reagent preparation that should be used in place of the one described in the EIA kit booklet₂

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BIBLIOGRAPHY

- ☞ **Chevenne D., Letailleur A., Trivin F., and Porquet D.,**
Effect of Hemolysis on the Concentration of Insulin in Serum Determined by RIA and IRMA
Clin. Chem., 44: 354 - 356, Feb 1998;
- ☞ **Sapin R.**
Insulin Immunoassays: Fast Approaching 50 Years of Existence and Still Calling for Standardization
Clinical Chemistry 53: 810-812, 2007

MATERIAL PROVIDED

- ☞ Insulinase Inhibitor cocktail 500 µL frozen, Ready to Use
- ☞ One vial of EIA buffer lyophilised
- ☞ One instruction booklet

MATERIAL REQUIRED BUT NOT PROVIDED

- ☞ EDTA collection tubes
- ☞ Precision micropipettes (20 to 1000 µL)
- ☞ Polypropylene tubes

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SAMPLE COLLECTION & PREPARATION

This protocol may be used to measure insulin in mouse/rat plasma or serum sample where hemolizes may occur. To do so, blood samples are collected in tubes containing EDTA. The samples are centrifuged at 1600 g for 20 minutes. Plasmas are collected and kept at -20°C until assay.

No prior extraction procedure is necessary to measure insulin in plasma samples.

- ☞ Insulinase Inhibitor cocktail
500 µL frozen, Ready to Use
Allow to collect 2.5 mL of sample and process one kit
- ☞ Inhibitor buffer
Dilute 250 µL of the inhibitor cocktail in 25 mL of EIA buffer provided in the EIA kit.

Afterwards, prepare mouse/rat insulin standards, quality control and samples as follows:

- ☞ Mouse/rat insulin standard
Reconstitute the vial with 1 mL of ultra pure water. Allow it to stand 5 minutes until the content is completely dissolved and then mix thoroughly by gentle inversion. Then, add 10 µL of the inhibitor cocktail. Prepare seven propylene tubes (for the seven other standards) and add 500 µL of the inhibitor buffer in each tube. Add 500 µL of the first tube (containing the first standard) to the second tube. Continue this procedure for the other tubes.

- ☞ Quality Control
Reconstitute one vial with 1 mL of ultra pure water. Allow it to stand 5 minutes until the content is completely dissolved and then add 10 µL of the inhibitor cocktail. Mix thoroughly by gentle inversion.

- ☞ Sample
Prior dispatching, add 10 µL of inhibitor cocktail for 1 mL of plasma (or 5 µL for 500 µL, 2 µL for 200 µL, etc..). If necessary, dilute the sample with the inhibitor cocktail buffer.

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