GYROS PROTEIN Technologies

Gyrolab® Assays

Insulin impurity Assay

INTRODUCTION

We have developed a three-step sandwich Gyrolab Assay to determine insulin in bioprocess samples. The assay has a broad analytical range with an approximate LOD of 20 pg/mL, LLOQ of 25 pg/mL, and ULOQ of 15 000 pg/mL. Use of this protocol on Gyrolab systems will reduce time to market and increase productivity while maintaining quality requirements.

ASSAY DESIGN

The assay was set up as a three-step sandwich assay with a biotinylated mouse monoclonal antibody (clone D3E7) as a capture molecule and an Alexa Fluor® 647-labeled mouse monoclonal antibody (clone D6C4) as a detection molecule.

anti-insulin/proinsulin

recombinant human insulin

anti-insulin/proinsulin

ASSAY PERFORMANCE

Dynamic range, accuracy and precision

A robust 4-log standard curve (Figure 1) was generated over three runs, achieving an assay range from 25 pg/mL to 15 000 pg/mL (Table 1). The Limit of Detection (LOD) was determined as a concentration corresponding to at least two standards deviations above the assay blank.

The inter-run precision (CV, Coefficient of Variation), established with QC samples over the assay range run in triplicate in three runs, was <20% (Table 2).

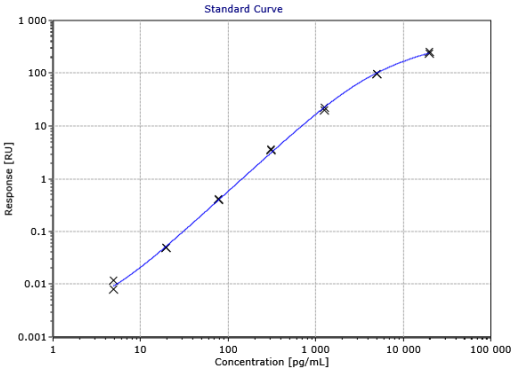

Figure 1 Typical standard curve

Table 1 Estimated Assay Range in based on three runs

LOD (pg/mL)	LLOQ (pg/mL)	ULOQ (pg/mL)
20	~ 25	~ 15 000

Table 2 Accuracy and precision data of QC samples, n= number of runs

QC	Expected Conc (pg/mL)	Average Measured Conc (pg/mL)	Inter Run CV% (n=3)	Average Intra Run CV% (n=3)	Average Total Error% (n=3)
LLOQ	25	25	15	10	19
LQC	50	51	7.9	6.7	11
MQC	500	546	7.9	6.8	16
HQC	10 000	10 459	8.1	4.8	10
ULOQ	15 000	16 840	6.9	4.6	17

MATERIALS AND METHODS

The assay was developed on Gyrolab xP using Gyrolab Bioaffy 1000 CD. The assay was set up using a 3-step Gyrolab method, 1000-3W-006-A. The capture molecule, a mouse monoclonal antibody (clone D3E7) from Fitzgerald Industries International was biotinylated according to the Gyrolab biotinylation protocol (Gyrolab User Guide) and used in a concentration of 100 µg/mL (~700 nM), diluted with PBS-T.

The detection antibody, labeled with Alexa Fluor® 647 according to the Gyrolab standard protocol (Gyrolab User Guide), was a mouse monoclonal antibody (clone D6C4) from Fitzgerald Industries International, diluted to 8.3 nM in Rexxip F. The assay standard used was the recombinant human insulin (30-AI51) from Fitzgerald Industries International. The standard and controls were diluted in Rexxip A.

Summary table

Capture	100 µg/mL biotinylated monoclonal anti-insulin/proinsulin, clone D3E7 from Fitzgerald Industries International in PBS-T Species reactivity: mouse, rat, human, cow and pig
Detection	Alexa Fluor 647-labeled monoclonal anti-insulin/proinsulin, clone D6C4 from Fitzgerald Industries International, 8.3 nM in Rexxip F Species reactivity: mouse, rat, human, cow and pig
Analyte	Recombinant human insulin (30-AI51 from Fitzgerald) in Rexxip A
CD-type	Bioaffy 1000 CD
Method	1000-3W-006-A
Wash buffer for needles	Wash buffer 1: PBS-T, wash buffer 2: Gyrolab wash buffer pH11
PMT-setting	1%
Expected dynamic range	Approximate 25-15 000 pg/mL

Recommendations

When developing this assay for a specific bioprocess, it is important to screen matrices and assess backgrounds. Parameters, such as LLOQ need to be validated in-house. Data given in this document should only be considered as a guidance.

For additional support contact your local Field Application Support