GYROS PROTEIN Technologies

Gyrolab® Assays

Erbitux® (cetuximab) PK Assay

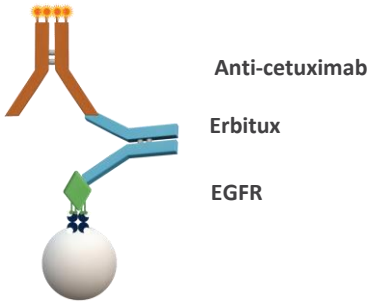
INTRODUCTION

Erbitux (cetuximab) is a cancer immunotherapy biopharmaceutical that belongs to the group of EGFR (epidermal growth factor receptor) inhibitors. Erbitux is a chimeric antibody of IgG1 isotype that binds to and inhibits the epidermal growth factor receptor.

We have developed a three-step bridging Gyrolab PK assay to determine Erbitux in human serum samples. A 10-fold sample dilution gives a broad analytical range with an approximate LLOQ of 20 ng/mL, and ULOQ of 2 000 ng/mL in neat serum. 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 bridging assay with biotinylated human EGFR as a capture molecule and a recombinant human anti-cetuximab labeled with Alexa Fluor® 647 as a detection molecule.



ASSAY PERFORMANCE

Dynamic range, accuracy and precision

A robust 3-log standard curve (Figure 1) was generated over three runs, achieving an assay range from 20 ng/mL to 2 000 ng/mL (Table 1). 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).

Table 1 Estimated Assay Range in neat serum, based on three runs

LLOQ (ng/mL)	ULOQ (ng/mL)
~ 20	~ 2 000

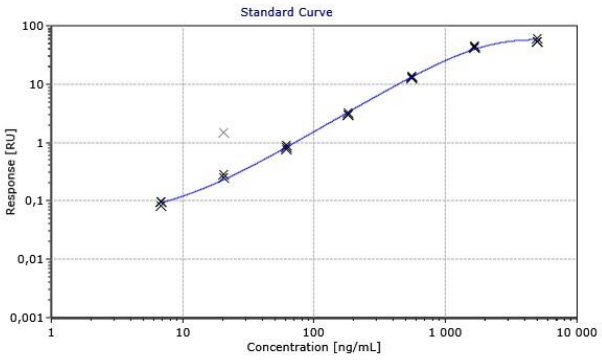


Figure 1 Standard curve in Rexxip H with 10% serum. Concentrations in neat serum

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

QC	Expected Conc (ng/mL)	Average Measured Conc (ng/mL)	Inter Run CV (%; n=3)	Average Intra Run CV (%; n=3)	Average Total Error (%; n=3)
1	20	22	15	6.4	18
2	75	75	13	6.9	17
3	200	193	5.5	2.6	7.6
4	600	631	6.2	4.5	10
5	2 000	2 537	5.8	5.4	32

Selectivity

Selectivity was established by spiking 20 ng/mL of the drug in human serum samples. All samples measured <LLOQ when analyzed unspiked.

Table 3 Selectivity spiked samples

Sample	Expected Conc (ng/mL)	Average Measured Conc (ng/mL)	CV (%)	Average Bias (%)
1	20	27	9.5	34
2	20	22	8.1	8.9
3	20	26	16	28
4	20	23	10	16
5	20	22	3.1	9.4
6	20	23	1.2	17
7	20	24	3.2	21
8	20	24	3.9	20
9	20	30	10	51
10	20	24	1.8	18

MATERIALS AND METHODS

The assay was developed on a Gyrolab xP system using Gyrolab Bioaffy 1000 CD. The assay was set up using a 3-step Gyrolab method with two wash solutions (1000-3W-006-A) and a 1% PMT setting. The assay buffer was Rexxip H with 10% human serum. Recombinant human EGFR (ab155639) from Abcam and BSA (Sigma, A3803) was biotinylated according the Gyrolab biotinylation protocol (Gyrolab User Guide) and used in a concentration of 70 nM, diluted with PBS-T containing 630 nM Biotin-BSA.

The detection antibody, labeled with Alexa Fluor® 647 according to the Gyrolab standard protocol (Gyrolab User Guide), was the anti-cetuximab (HCA221) from Bio-Rad, diluted to 35 nM in Rexxip F. The assay standard used was the chimeric cetuximab (Erbitux) from Merck. The standard was prepared in human serum diluted in Rexxip H.

Summary table

Capture	70 nM biotinylated EGFR (Abcam, # ab155639) + 630 nM biotinylated BSA in PBST
Detection	35 nM Alexa Fluor 647 labeled anti-cetuximab (BioRad, # HCA221) in Rexxip F
Analyte	Erbitux (MSD) in Rexxip H with 10% human serum
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 pH 11
PMT-setting	1%
Expected dynamic range	Approximately 20 - 2 000 ng/mL in neat human serum

Recommendations

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

For additional support contact your local Field Application Support