GYROS PROTEIN Technologies

Gyrolab® Assays

Herceptin® (trastuzumab) PK Assay

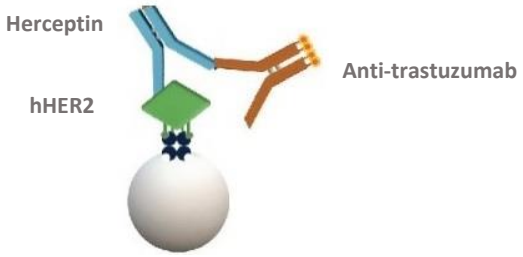
INTRODUCTION

Herceptin (trastuzumab) is a biopharmaceutical that is used in breast cancer immunotherapy. Herceptin is a humanized antibody of IgG1 isotype that targets HER2, inducing an immune-mediated response that causes internalization and downregulation of HER2. HER2 is overexpressed in 20-30% of early stage breast cancers.

We have developed a three-step bridging Gyrolab PK assay to determine herceptin in human serum samples. An MRD of 1:20 gives a broad analytical range with an approximate LLOQ of 200 ng/mL, and ULOQ of 200 000 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 HER2 as a capture molecule and recombinant human anti-trastuzumab labeled with Alexa Fluor® 647 as 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 200 ng/mL to 200 000 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 generally <20% (Table 2).

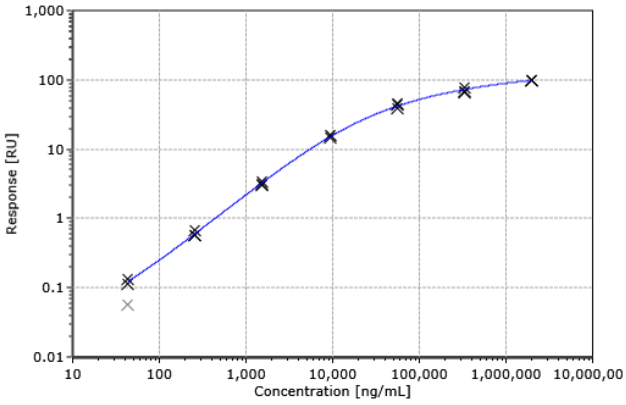


Figure 1 Standard curve in Rexxip H with 5% serum. Concentrations in neat serum

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

LLOQ (ng/mL)	ULOQ (ng/mL)
~ 200	~ 200 000

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	200	208	17	13	20
2	1 500	1 480	6.6	6.8	8.2
3	8 000	8 016	5.1	4.9	6.9
4	40 000	39 048	8.1	5.2	11
5	200 000	198 509	21	19	29

Selectivity

Selectivity was established by spiking 200 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	200	192	8.1	-4.0
2	200	233	11	17
3	200	195	2.0	-2.5
4	200	206	16	2.8
5	200	206	17	3.0
6	200	219	5.8	9.6
7	200	241	4.8	21
8	200	194	9.4	-3.2
9	200	198	16	-1.0
10	200	213	3.6	6.6

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 5% PMT setting. The assay buffer was Rexxip H with 5% human serum. Human HER2 from Sino Biological (10004-H08H) was biotinylated as detailed below and used at a concentration of 700 nM, diluted with PBS-T.

Recombinant HER2 protein as supplied was reconstituted to 1 mg/mL with deionized water. The reconstituted protein was then reacted with 12 molar equivalents of Biotin-XX, SE (Thermo Fisher Scientific, B1606) as a 4 mg/mL solution freshly prepared using DMSO, and the resulting mixture vortexed briefly, before being roller-mixed for 1 hour at 20°C, in the dark. After, the conjugate was purified by desalting into 50 mM phosphate 150 mM NaCl pH 6.7 buffer via zeba 0.5 mL spin desalting column, and UV analyzed to determine product concentration.

The detection antibody, supplied labeled with Alexa Fluor® 647, was recombinant rabbit anti-trastuzumab FAB95471R from R&D Systems, diluted to 35 nM in Rexxip F. The assay standard was humanized IgG1 monoclonal antibody trastuzumab from Roche. The standard was prepared in 5% human serum diluted in Rexxip H.

Summary table

Capture	700 nM biotinylated HER2 (10004-H08H, Sino Biological)
Detection	Alexa Fluor 647 labeled anti-trastuzumab (FAB95471R, R&D Systems) 35 nM in Rexxip F
Analyte	Herceptin (Roche) in Rexxip H with 5% 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	5%
Expected dynamic range	Approximately 200-200 000 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