GYROS PROTEIN Technologies

Gyrolab® Assays

Rat Erythropoietin (EPO) Assay

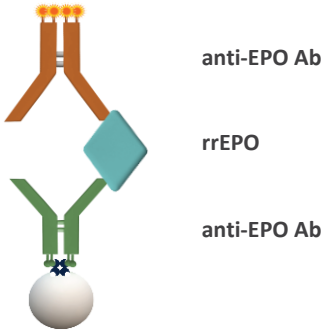
INTRODUCTION

Erythropoietin (Hematopoietin, MVCD2, EP or EPO) is a secreted cytokine hormone involved in erythrocyte maturation. Mature rat EPO consist of 166 amino acids (N-term ALA-27) and contains three N-glycosylation sites. The functional glycosylated protein has a molecular weight of 30-34 kDa whereas the deglycosylated form is 18 kDa. EPO is produced in the kidneys and liver under hypoxic conditions.

Rat EPO share 79% respectively 95% sequence homology to human and mouse EPO. The reagents used in this assay are cross-reactive to these species.

ASSAY DESIGN

The assay was set up as a three-step sandwich assay with a biotinylated anti-EPO antibody as a capture molecule and an Alexa Fluor® 647 labelled anti-EPO antibody as a detection molecule. Recombinant rat EPO (rrEPO) was used as standard material.



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 5 pg/mL to close to 20 000 pg/mL using the present assay set up (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 duplicate in three runs, was <20% (Table 2).

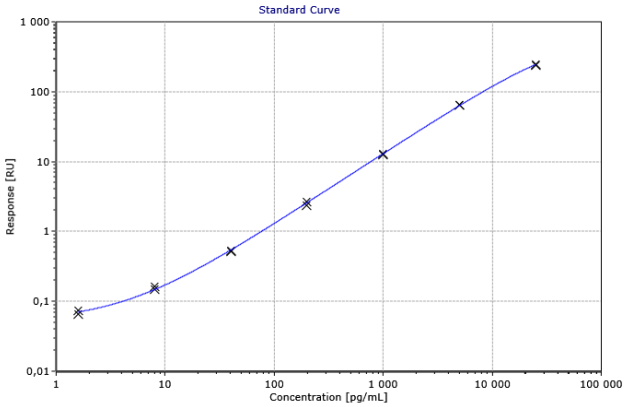


Figure 1 Standard curve in Rexxip A

Table 1 Estimated Assay Range, based on three runs

Assay range	LOD (pg/mL)	LLOQ (pg/mL)	ULOQ (pg/mL)
On plate	~3	~5	~20 000
In matrix, diluted 1:2	~6	~10	~40 000

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

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)
5	5.3	13	7.7	25
10	11	7.4	4.7	13
50	49	2.5	6.4	9.0
200	201	4.2	2.7	5.5
1 000	1 031	3.4	4.2	7.6
5 000	5 217	1.3	3.3	7.7
15 000	16 784	2.3	5.2	17
20 000	21 722	2.5	3.9	13

Parallelism

Parallelism was examined by diluting serum samples from three individuals and one serum pool (Sprague Dawley) containing detectable endogenous levels of EPO. The samples were serially diluted in Rexxip A-max (1:2 dilution) and Rexxip A (1:4 - 1:32) (Table 3). It is recommended that the end user performs parallelism assessment on rat serum samples with elevated endogenous levels of EPO.

Table 3 Parallelism. Each dilution analyzed in triplicate

Sample	Dilution Factor	Calculated Conc (pg/mL)	CV (%)	Recovery % of lowest dilution
1	1	111	3.4	100
	2	112	1.8	101
	4	114	5.5	102
	8	105	5.6	94
	16	114	16	102
	32	<LLOQ	-	-
2	1	117	4.9	100
	2	117	3.9	101
	4	110	7.9	94
	8	117	8.6	100
	16	118	9.9	101
	32	<LLOQ	-	-
3	1	121	2.1	132
	2	91	3.3	100
	4	93	3.4	101
	8	86	12	95
	16	<LLOQ	-	-
Serum pool	1	83	3.8	100
	2	81	2.8	98
	4	81	1.2	97
	8	77	3.2	93
	16	<LLOQ	-	-

MATERIALS AND METHODS

The assay was developed on Gyrolab xP systems using Gyrolab Bioaffy 1000 HC CD. The assay was set up using a three-step method with two wash solutions (1000HC-3W-011-A) and a 1% PMT setting. The assay buffer was Rexxip A. Three out of four tested serum samples could be analyzed as neat.

The matched antibody pair from the DuoSet mouse EPO ELISA (R&D Systems, DY959) was used for this assay (capture and detection reversed). Biotinylated anti-EPO antibody (part 841005) was reconstituted to 0.5 mg/mL in PBS and used in a concentration of 100 µg/mL, diluted in PBS-T. The unlabeled anti-EPO antibody (part 841004) was reconstituted to 1 mg/mL in PBS, followed by Alexa Fluor® 647 labeling according to the Gyrolab standard protocol (Gyrolab User Guide). The final assay concentration was 10 nM in Rexxip F.

Recombinant rat EPO (R&D Systems, 1306-RE) was reconstituted in PBS + 0.2% BSA. The standard curve was prepared in Rexxip A.

Summary table

Capture	100 µg/mL biotinylated rat anti-mouse EPO (R&D Systems, DY959, part 841005) in PBS-T
Detection	Alexa Fluor 647-labeled rat anti-mouse EPO (R&D Systems, DY959, part 841004), 10 nM in Rexxip F
Analyte	Recombinant Rat EPO in Rexxip A (R&D Systems, 1306-RE)
CD-type	Bioaffy 1000 HC CD
Method	1000HC-3W-011-A (available for download at Gyrolab User Zone)
Wash buffer for needles	Wash buffer 1: PBS-T Wash buffer 2: Gyrolab Wash Buffer pH11
PMT-setting	1%
Expected dynamic range	Approximate 5 pg/mL to 20 000 pg/mL (approximate 10-40 000 pg/mL in neat rat serum, dilution 1:2)

Recommendations

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

For additional support contact your local Field Application Support