GYROS PROTEIN Technologies

Gyrolab® Assays

Mouse Erythropoietin (EPO) Assay

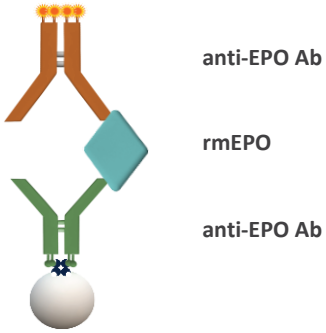
INTRODUCTION

Erythropoietin (Hematopoietin, MVCD2, EP or EPO) is a secreted cytokine hormone involved in erythrocyte maturation. Mature mouse 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.

Mouse EPO share 78% and 95% sequence homology to human and rat EPO respectively. 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 mouse EPO (rmEPO) 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 30 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 standard 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).

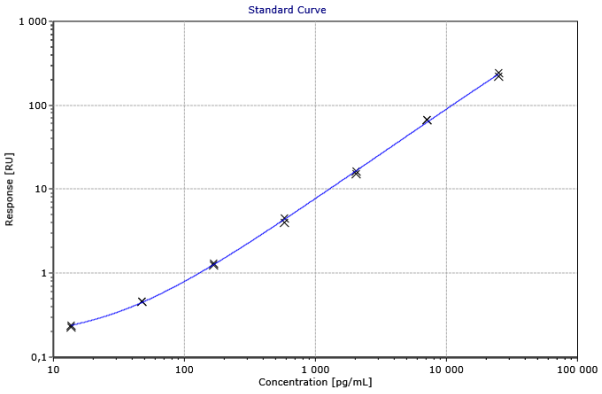


Figure 1 Standard curve in Rexxip A

Table 1 Estimated Assay Range, based on three runs

Assay range	LOD (pg/mL)	LLOQ (pg/mL)	ULOQ (pg/mL)
On plate	<20	~30	~20 000
In serum, diluted 1:2	<40	~60	~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)
30	32.4	8.7	17	25
100	120	6.4	4.6	24
1 000	1 153	4.7	2.9	18
15 000	15 868	1.7	1.9	7.6
20 000	20 323	2.0	3.9	5.9

Parallelism

Parallelism was examined by diluting serum samples from two individuals and one serum pool (BALB/c) containing detectable endogenous levels of EPO. The samples were serially diluted in Rexxip A (Table 3). Some serum sample could be analyzed as neat (data not shown). It is recommended that the end user performs parallelism assessment on mouse serum samples with elevated endogenous levels of EPO.

Table 3 Parallelism. Each dilution analyzed in triplicate. Recovery was calculated as percentage compared to the lowest dilution

Sample	Dilution Factor	Calculated Conc (pg/mL)	CV (%)	Recovery (%)
1	2	407	3.6	100
	4	416	5,5	102
	8	375	12	92
	16	<LLOQ	-	-
2	2	675	3,5	100
	4	658	1,9	98
	8	587	4,3	87
	16	627	2,6	93
	32	<LLOQ	-	-
Serum pool	2	1 528	1,7	100
	4	1 550	3,1	101
	8	1 645	6,5	108
	16	1 698	6,2	111
	32	1434	6.8	94
	64	<LLOQ	-	-

MATERIALS AND METHODS

The assay was developed on Gyrolab xP and Gyrolab xPand 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 5% PMT setting. The assay buffer was Rexxip A.

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 from the DuoSet) 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 from the DuoSet) 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 mouse EPO (part 841006 from the DuoSet) 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 Mouse EPO in Rexxip A (R&D Systems, DY959, part 841006)
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	5%
Expected dynamic range	Approximate 30 - 20 000 pg/mL (approximate 60 - 40 000 pg/mL in neat mouse 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