

Gyrolab® Assays

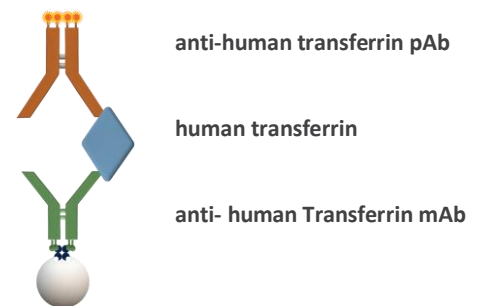
# Human Transferrin Impurity Assay

## INTRODUCTION

We have developed a three-step sandwich Gyrolab Assay to determine human transferrin in bioprocess samples. The assay has a broad analytical range with an approximate LOD of 0.03 ng/mL, LLOQ of 0.1 ng/mL, and ULOQ of 150 ng/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 biotinylated mouse anti-human transferrin, clone 2A2, as a capture molecule and alexa-647 labelled polyclonal goat anti-human Transferrin as a detection molecule.



## 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 0.1 ng/mL to 150 ng/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 3 runs, was <20% (Table 2).

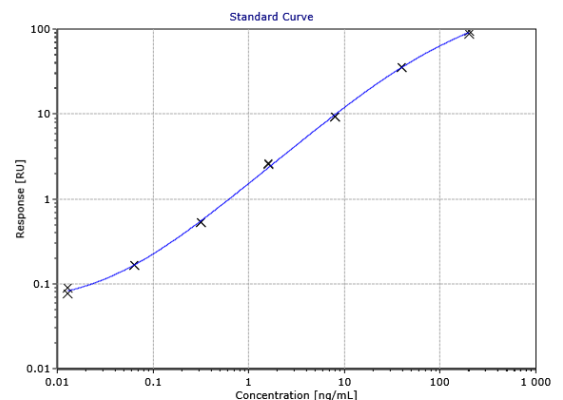


Figure 1 Typical standard curve

Table 1 Estimated Assay Range in based on three runs

LOD (ng/mL)	LLOQ (ng/mL)	ULOQ (ng/mL)
~0.03	~0.1	~150

Table 2 Accuracy and precision data of QC samples, 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)
LLOQ	0.10	0.11	9.8	6.4	16
LQC	0.20	0.21	7.1	3.6	10
MQC	2.0	2.1	3.4	2.0	9.0
HQC	100	99	5.8	5.1	8.3
ULOQ	150	158	7.1	6.6	12

## 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. Mouse anti-human transferrin (clone 2A2, HyTest Ltd) was biotinylated according to the Gyrolab biotinylation protocol (Gyrolab User Guide) and used in a concentration of 700 nM, diluted in PBS-T.

The detection antibody, labeled with Alexa Fluor®-647 according to the Gyrolab standard protocol (Gyrolab User Guide), was a goat anti-human transferrin pAb (#A80-128A, Bethyl Laboratories, Inc) diluted to 6.25 nM in Rexpip F. The assay standard used was human transferrin (#T3309, Sigma). The standard and controls were prepared in Rexpip A.

### Summary table

<b>Capture</b>	100 µg/mL biotinylated mouse anti-human transferrin, clone 2A2 from HyTest Ltd, diluted in PBS-T
<b>Detection</b>	Alexa Fluor 647-labeled goat anti-human transferrin (A80-128A) from Bethyl Laboratories, Inc, 6.25 nM in Rexpip F
<b>Analyte</b>	Human transferrin, Product Number T3309, Sigma, diluted in Rexpip A
<b>CD-type</b>	Bioaffy 1000 CD
<b>Method</b>	1000-3W-006-A
<b>Wash buffer for needles</b>	Wash buffer 1: PBS-T, wash buffer 2: Gyrolab wash buffer pH11
<b>PMT-setting</b>	1%
<b>Expected dynamic range</b>	Approximate 0.1-150 ng/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

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