

Gyrolab® Assays

# Neulasta® (pegfilgrastim) PK Assay

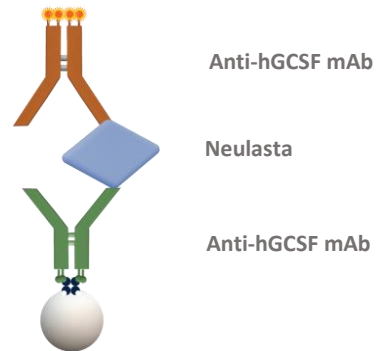
## INTRODUCTION

Neulasta (pegfilgrastim) is a PEGylated form of the recombinant human granulocyte colony-stimulating factor (hGCSF) analog filgrastim. It serves to stimulate the production of white blood cells and is used to decrease the incidence of infection, by treating neutropenia, which is a lack of certain white blood cells caused by cancer chemotherapy.

We have developed a three-step sandwich Gyrolab PK assay to determine Neulasta in human serum samples. An MRD of 1:4 gives a broad analytical range with an approximate LLOQ of 4 ng/mL, and ULOQ of 8 000 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 sandwich assay with biotinylated mouse anti-hGCSF mAb as a capture molecule and rat anti-hGCSF mAb labeled with Alexa Fluor® 647 as a detection molecule.



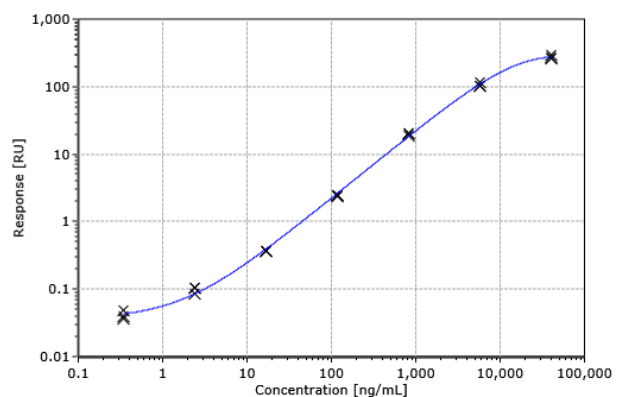
## ASSAY PERFORMANCE

### Dynamic range, accuracy and precision

A robust 3-log standard curve (Figure 1) was generated over three runs, achieving an assay range from 4 ng/mL to 8 000 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).

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

LLOQ (ng/mL)	ULOQ (ng/mL)
~ 4	~ 8 000



**Figure 1** Standard curve in REXXIP H with 25% 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	4	3.5	14	14	25
2	20	17	16	4.1	19
3	80	75	21	3.5	20
4	800	833	9.8	5.6	14
5	8000	7755	7.6	5.6	11

### Selectivity

Selectivity was established by spiking 4 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	4	3.2	2.8	-19
2	4	3.3	4.0	-17
3	4	4.3	12	6.5
4	4	3.4	12	-15
5	4	3.5	12	-12
6	4	4.2	2.7	4.7
7	4	3.9	4.5	-2.7
8	4	4.5	18	13
9	4	4.5	11	12
10	4	4.1	17	2.4

## 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 25% human serum. Mouse anti-human GCSF from Abcam (clone 5D7) was biotinylated according to the Gyrolab standard protocol (Gyrolab User Guide) and used at a concentration of 700 nM, diluted with PBS-T.

The detection antibody labelled with Alexa Fluor 647 according to the Gyrolab standard protocol (Gyrolab User Guide), was rat anti-human GCSF (clone BVD13-3A5) from BioRad, diluted to 35 nM in REXXIP F. The assay standard used was the PEGylated recombinant GCSF protein pegfilgrastim from Amgen. The standard was prepared in 25% human serum diluted in REXXIP H.

## Summary table

<b>Capture</b>	700 nM biotinylated anti-human GCSF (clone 5D7, Abcam, ab9818)
<b>Detection</b>	Alexa Fluor 647 labeled anti-human GCSF (clone BVD13-3A5, BioRad, 1012701) 35 nM in Rexpip F
<b>Analyte</b>	Neulasta (Amgen) in Rexpip H with 25% human serum
<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 pH 11
<b>PMT-setting</b>	1%
<b>Expected dynamic range</b>	Approximately 4-8000 ng/mL in neat matrix

## 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

Gyrolab and Rexpip are registered trademarks and Gyros, Gyrolab xPlore, Gyroplex, Bioaffy and Gyros logo are trademarks of Gyros Protein Technologies Group. All other trademarks are the property of their respective owners. Products and technologies from Gyros Protein Technologies are covered by one or more patents and/or proprietary intellectual property rights. All infringements are prohibited and will be prosecuted. Please contact Gyros Protein Technologies AB for further details. Products are for research use only. Not for use in diagnostic procedures. © All infringements are prohibited and will be prosecuted. Please contact Gyros Protein Technologies AB for further details. Products are for research use only. Not for use in diagnostic procedures. © Gyros Protein Technologies AB 2020. D0034759/-