Instruction For Use

Gyrolab® Protein A Kit

Product number
P0020456

Product Name
Gyrolab Protein A Kit for MabSelect SuRe Ligand

1. Product Description

Gyrolab Protein A Kit for MabSelect™ SuRe™ Ligand is a ready-to-use kit for analysis of leached Protein A from MabSelect SuRe affinity chromatography columns. This document describes how to perform the analysis using the kit, with Gyrolab® Mixing CD 96 and the ready-made reagents provided.

Gyrolab® Protein A Kit is for research only and not intended for diagnostic use.

2. Introduction

Protein A and Protein A derivatives such as MabSelect SuRe are commonly used as affinity ligands in the purification of therapeutic monoclonal antibodies. Protein A can, however, leach from the chromatography resin and co-elute with the therapeutic antibody product and, in addition, bind to immunoglobulins, increasing the risk of adverse reactions. Quantifying Protein A impurity levels during purification is therefore a regulatory requirement.
Gyrolab Protein A Kit is intended for quantification of Protein A ligands in relation to a defined concentration of IgG. Results are typically reported as 'parts-per-million' (ppm), i.e. the ratio of Protein A ligand in relation to the amount of IgG present in the sample.
Gyrolab Protein A Kit for MabSelect SuRe includes MabSelect SuRe as standard and can be used to detect MabSelect™, MabSelect™ SuRe™ and MabSelect™ SuRe™ LX – ligands. Accurate quantification of protein A ligands other than MabSelect SuRe in real samples may require using a standard that matches the Protein A from the affinity column material.

3. Assay Principle

Measuring Protein A directly in bioprocess samples may underestimate the impurity level since IgG can occupy relevant Protein A epitopes, preventing accurate protein A quantification. Gyrolab Protein A Kit utilizes an acid dissociation principle which involves treatment of the sample with acid to dissociate Protein A from IgG, prior to quantification of Protein A in the presence of IgG, using a standard sandwich immunoassay principle.

In the completely automated Gyrolab method for quantifying residual Protein A ligands, samples are first treated with a low pH buffer in a Gyrolab Mixing CD 96 to dissociate Protein A and the IgG product. Following a pH adjustment step, Protein A is measured using the sandwich immunoassay.

The sandwich immunoassay is prepared by introducing a biotinylated capture reagent into the streptavidin-coated affinity column. The dissociated samples are passed through the affinity-capture column where Protein A is captured, a fluorescently labeled detection antibody is added and samples are quantified. The results are analyzed using 21 CFR part 11-compliant Gyrolab Evaluator software.

Gyrolab method description

To determine the concentration of MabSelect SuRe ligand in the presence of IgG, the Gyrolab method involves acidification of samples in the mixing chamber (Figure 1). The acid conditions used in the assay have been selected to make any Protein A ligand in the sample fully accessible in the assay while maintaining assay performance. The sample is acidified at two pH levels in the mixing chamber:

1. A lower pH **(Reagent D)** is used to dissociate MabSelect SuRe from the IgG molecule, and
2. The pH of the acidified sample is adjusted using **Reagent E** to a pH compatible with the capture reagent.

The dissociated sample is then passed through to the capture column. MabSelect SuRe is provided as a standard (**Reagent C**) in the kit.

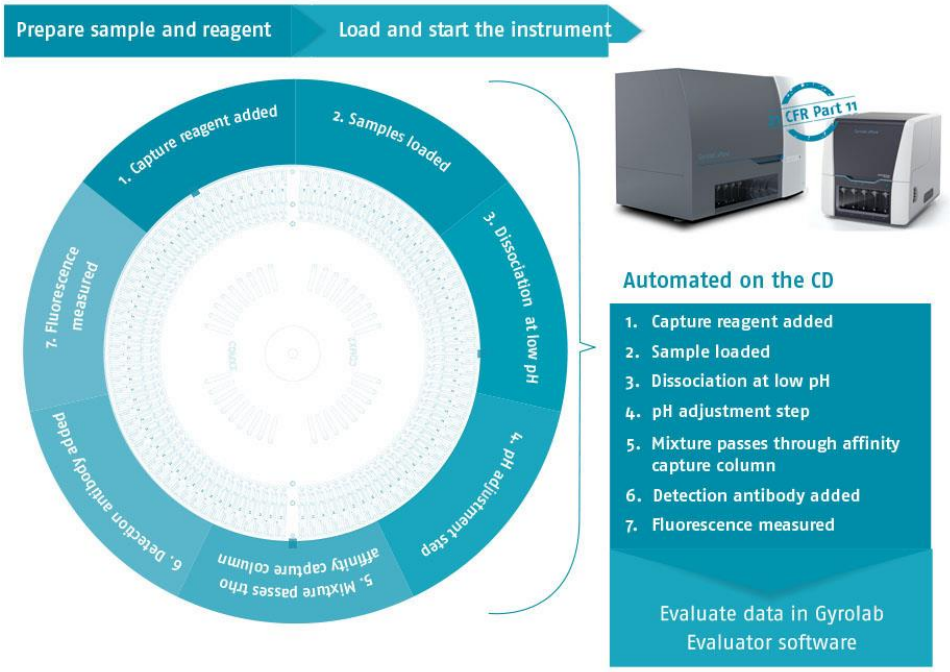

Figure 1. *Gyrolab method for determination of Protein A residuals.*

4. Limitations

Partial Use of Kits

For single CD kits we recommend no more than two times partial use of kit reagents and CD that have been removed from its pouch. Use within one week.

Matrix effect

Certain sample matrices may interfere with the assay and to test for this we recommend that all samples are diluted at several dilutions in the Sample Dilution Buffer provided.

Assay Qualification

We recommend that you qualify or validate the assay for its intended use to ensure that the assay protocol yields acceptable performance, such as accuracy and precision, before using it to report residual MabSelect SuRe.

5. Storage and Stability

Reagents

All reagents must be stored at +4 to +8 °C to maintain functionality. Gyrolab Wash Buffer pH 11 can be stored at 4-28°C.

Unopened CD package

Refrigerate at +4 to +8 °C, pouch unopened.

Opened CD package

CDs must be used within one week after opening. Return partially used CDs to original CD packaging. Re-seal. Store dark, dry and at ambient temperature.

6. Reagents, Methods & Materials

Kit Components	Product #

Content

Gyrolab Protein A Kit Reagents: Contents see below

Gyrolab CD: One (1) Gyrolab Mixing CD 96	P0020455
Wash solution for needles: Gyrolab Wash Buffer pH 11	P0020087
96-well plate: Three (3) 0.2 mL skirted PCR plate	P0004861
Foil: Three (3) Microplate Foil	P0003313

Gyrolab Protein A Kit Reagents

Reagent A: Capture Reagent, Biotinylated Anti-Protein A, Ready to use solution, 70 µL

Reagent B: Detection Reagent, Fluorophore-labeled Anti-Protein A, Ready to use solution, 70 µL

Reagent C: Standard MabSelect SuRe, 1000 ng/mL, 50 µL

Reagent D: Acid Dissociation Buffer 1, 500 µL

Reagent E: Acid Dissociation Buffer 2, 500 µL

Reagent F: Acidic Wash Buffer, 500 µL

Reagent G: Wash Buffer, 1000 µL

Reagent H: Sample Dilution Buffer, 25 mL

Other Materials and Components required but not provided

- Gyrolab system with Gyrolab Control v.5.4 or later
- Gyrolab Evaluator v.3.3 or later
- PBS-T wash solution for Gyrolab wash stations and pump liquid – see Gyrolab User guide
- Pipettes or pipetting equipment with disposable polypropylene tips
- Disposable polypropylene test tubes
- Lab centrifuge
- Vortex mixer
- Distilled or deionized water
- Filtering equipment with 0.22 or 0.45 µM filters

CD design

For instruments with Gyrolab Control software versions older than 7.1.3, a new CD design, “Gyrolab Mixing CD 96”, must be imported prior to the first analysis, please see Gyrolab User Zone at www.gyrosproteintechnologies.com/gyrolab-user-zone.

Gyrolab Method

The Gyrolab method **‘Protein A kit method v3’** must be installed on the instrument being used for analysis. If the method is not present in the database it must be installed. The method can be downloaded from Gyrolab User Zone, www.gyrosproteintechnologies.com/gyrolab-user-zone.

Please observe that for Gyrolab systems with software versions lower than 8.0, the method requires the use of two microtiter plates. These should be loaded with:

- Standard, controls and samples (one or several plates depending on number of samples)
- Reagents and Wash buffers

respectively.

Gyrolab Protein A kit run v3 template run

To simplify setting up a run design, a template run for designing a new run can be downloaded from Gyrolab User Zone at www.gyrosproteintechnologies.com/gyrolab-user-zone.

Check if the Gyrolab Protein A kit Run v3 is present in the database. If not open the Administrator tool and import the Run ‘**Gyrolab Protein A kit Run v3’.** The method will be imported automatically together with the Run. Close the admin tool. For more detailed instruction see User Guide.

Note: if Gyrolab Protein A kit run v3 is installed the ‘Protein A kit method v3’ is automatically installed.

7. Preparation of reagents

Note! Briefly spin all vials in a micro-centrifuge before opening to collect all liquid at the bottom.
Note! Ensure that pipetting routines are designed to minimize contamination, e.g. use clean pipettes and new tips. Contamination may reduce the utility/shelf life of the reagent.

Reagents and standard curve should be prepared according to Tables 1–2 below. We recommend that at least one QC sample has a concentration close to the Lower Limit of Quantitation (LLOQ) and is prepared in sample matrix with your product antibody. See the example in Table 2.

Capture Reagent

The Capture Reagent (**Reagent A**) is ready-to-use and is transferred directly to the microplate according to the Gyrolab Control Loading List

Detection Reagent

The Detection Reagent (**Reagent B**) is ready-to-use and is transferred directly to the microplate according to the Gyrolab Control Loading List.

Reagent D, E ,F

The acidic buffers are ready to use and is transferred directly to the microplate according to Gyrolab Control Loading list.
Note that Reagent F is placed in two microplate wells and named F and F2.

8. Standard curve

Table 1 shows an example of how to prepare a standard curve assuming the stock solution of MabSelect SuRe (**Reagent C**) is 1000 ng/mL. We recommend that the MabSelect SuRe standard curve is in the range of 0.03 to 120 ng/mL. Dilute the standard curve in Sample Dilution Buffer (**Reagent H**). Prepare the dilutions in polypropylene tubes and vortex the tubes throughout the dilution series before transferring to the PCR plate.

Table 1. Example of a standard curve for MabSelect SuRe

	Conc. MabSelect SuRe [ng/mL]	Volume MabSelect SuRe stock [µL]	Volume higher Std conc. [µL]	Volume Reagent H [µL]
Std 1	120	6		44
Std 2	30		10	30
Std 3	7.5		10	30
Std 4	1.88		10	30
Std 5	0.47		10	30
Std 6	0.12		10	30
Std 7	0.03		10	30
Blank	0		0	30

QC samples

Table 2 shows an example of how to prepare QC samples assuming the stock solution of MabSelect SuRe (**Reagent C**) is 1000 ng/mL. Prepare the dilutions in polypropylene tubes. Vortex before transferring to the PCR plate.

Table 2. *Example of dilutions of QC samples*

	Conc. MabSelect Sure [ng/mL]	Volume MabSelect SuRe stock [µL]	Volume higher QC conc. [µL]	Volume Reagent H [µL]
	100	5		45
QC1	50		25	25
QC2	5		10	90
QC3	0.5		10	90

9. Establishing the optimal assay performance conditions

To characterize and optimize the assay run conditions for a new IgG molecule, we recommend that you run a serial dilution of samples, unspiked and spiked with a known concentration of MabSelect SuRe.

Start by diluting the sample down to an IgG concentration of **10** mg/mL with sample dilution buffer (**Reagent H**). Mix the sample 1:1 with a MabSelect SuRe spiked sample dilution buffer containing 10 ng/mL MabSelect SuRe (**Reagent C**) to obtain a sample with 5 mg/mL IgG and 5 ng/mL MabSelect SuRe to be used as stock. Prepare the serial dilution series according to Table 3 and Table 4, below, with spiked and unspiked sample. Prepare the dilutions in polypropylene tubes. Vortex before transferring to the PCR plate.

It is recommended that the MabSelect SuRe spike concentration is similar to the expected MabSelect SuRe concentration, typically 5-10 ng/mL will be sufficient to obtain accurate recovery results.

Table 3. *Example of serial dilution of spiked samples*

Serial Dilution	**Conc. IgG [mg/mL]**	**Spiked MabSelect SuRe conc [ng/mL]**	**Volume higher sample conc. [µL]**	**Volume Reagent H [µL]**
1	5	5	40 (stock)	
1:2	2.5	2.5	20	20
1:4	1.25	1.25	20	20
1:8	0.625	0.625	20	20

Table 4. *Example of serial dilution of unspiked samples*

Serial Dilution	**Conc. IgG [mg/mL]**	**Volume higher sample conc. [µL]**	**Volume Reagent H [µL]**
1	5	20 (10 mg/mL)	20
1:2	2.5	20	20
1:4	1.25	20	20
1:8	0.625	20	20

Determine the highest IgG concentration that passes your recovery specification for the spiked + unspiked samples. Tables 3 and 4 demonstrates how to perform spike recovery and dilution linearity experiments and can be modified according to preference.
When optimal assay conditions have been established, follow the protocol below.

10. Sample preparation

Dilute the sample to an IgG concentration determined in the serial dilution run using **Reagent H** (Sample dilution Buffer). Note that to ensure optimal analytical performance, minimum dilution with **Reagent H** is 1:3 (1 part sample + 2 parts Reagent H).

Preparation of Gyrolab Wash Buffer pH 11

1. Dissolve one package (10 g) of Gyrolab Wash Buffer pH 11 powder in 1 L of deionized or distilled water for a one-CD run or dissolve two packages (20 g) of Gyrolab Wash Buffer pH 11 powder in 2 L of deionized or distilled water for a five-CD run.
2. Filter the solution through a 0.22 or 0.45 µm filter.

Note!

- Prepare fresh wash solution weekly
- For research use only

Important Notice regarding wash solution volumes required when running the Protein A Kit method:
The needle wash procedures in the Protein A kit method have carefully optimized to minimize carry-over from high concentrations of IgG, typical for samples to be analyzed for Mab Select SuRe leachable. The consumption of wash buffer solutions 1 and 2 used by the method are therefore higher than other Gyrolab methods.

Required volume

- 1000 mL for 1 CD
- 2000 mL for 2-3 CDs
- 3000 mL for 4-5 CDs

Please ensure the use of appropriate bottles to ensure that the wash solution volumes are sufficient for the run.

11. Data Analysis

The data is evaluated in Gyrolab Evaluator version 3.5 or later. Open the run and select ‘Quantification’. In ‘Analysis Setup’ default settings are recommended:

- do not include blanks in curve fitting
- five parameter logistic curve
- weight on response
- limit of detection factor: 2

Select ‘LOQ matrix’: diluted and enter 0.1 ng/mL as LLOQ and 100 ng/mL as ULOQ (or values established in-house – these are preliminary assay performance characteristics). Acceptance criteria for %CV and bias can be added to highlight values outside these criteria.

A concentration of MabSelect SuRe ligands is usually expressed in relation to the IgG concentration (parts per million or ppm).

12. Performance characteristics

12.1 Standard curve

A typical standard curve is shown in Figure 2. The standard curve has been repeated three times and the curves are shown together in the same figure.

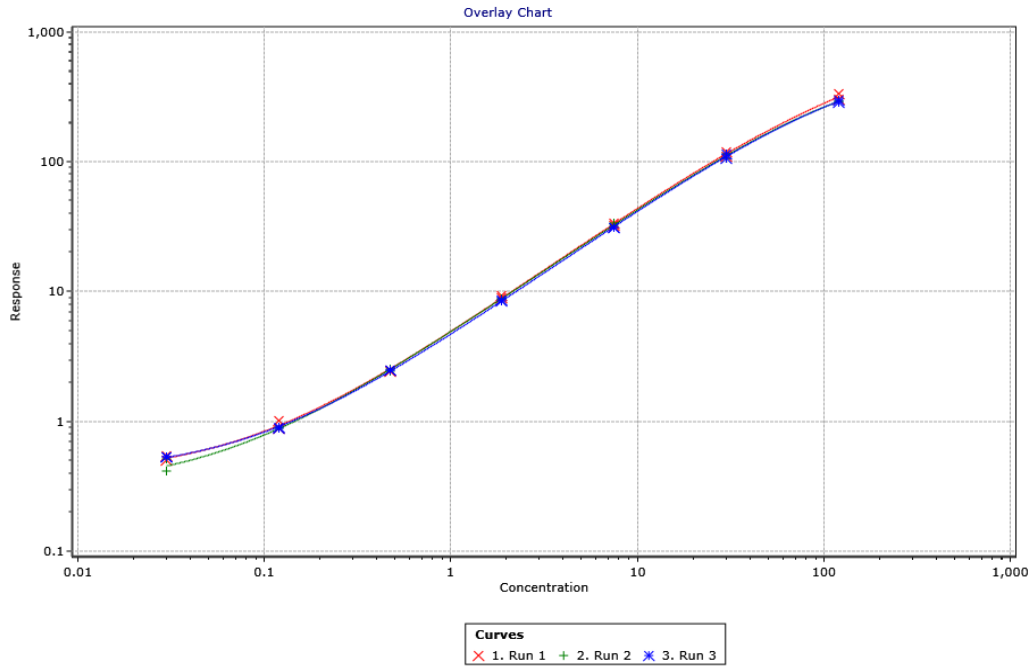

Figure 2. *Typical standard curves generated from three consecutive runs using MabSelect SuRe as standard demonstrates linearity and reproducibility.*

12.2 Sensitivity

Typical LLOQ for MabSelect Sure is presented in Table 5. LLOQ and ULOQ were established in six runs as concentrations where Total Error (%CV + absolute %RE) < 30%, see Table 6.
The LLOQ should be established for each IgG molecule and for matrices from all steps of the bioprocess that is evaluated in the assay.

Table 5. *Typical assay characteristics for Gyrolab Protein A Kit*

	MabSelect SuRe	
Assay Characteristics	(ng/mL)	(ppm)
LLOQ (5 mg/mL IgG*)	~0.1	~0.02

*Commercially available IgG molecule (MAb)

12.3 Precision

Intra and inter precision data are presented in Table 6 for and MabSelect SuRe. The precision data for five curves are shown in table 6.
The intra CV has been determined for duplicates of each control sample and the inter CV precision has been determined with duplicates on 5 CDs.

Table 6. *Accuracy and precision data for five QC samples*

Sample	Exp conc (ng/mL)	Av. Measured conc (ng/mL)	Intra-run CV (%)	Inter-run CV (%)	TE range (%)
QC 1	80	80.6	3.00%	6.70%	4.1 - 14.9
QC 2	2	1.9	7.4%	4.90%	2.0 - 13.7
QC 3	0.3	0.3	7.5%	11.00%	5.0 - 23.8
QC 4	0.15	0.2	14.3%	17.80%	18.1 -32.9
QC 5	0.1	0.1	9.1%	14.50%	12.5 - 28.1

12.4 Dilutional Linearity and Spike Recovery

Table 8 shows spike recovery experiments where MabSelect SuRe at 5 ng/mL were spiked into different clinical grade therapeutics at 20-60 mg/mL concentrations followed by further dilution and analysis for protein A using Gyrolab Protein A kits for MabSelect SuRe to determine spike recovery.

Table 8. *Spike Recovery data*

Sample	Dilution	SPIKED		UNSPIKED		Spiked conc [ng/ml]	MabSSR conc [ng/ml]	Spike recovery	ppm	AVG MabSSR conc [ng/ml]	ppm
		Calc conc [ng/ml]	CV Conc	Calc conc [ng/ml]	CV Conc						
IgG 1 20 mg/ml	4	7.2	0.5	2.7	4.3	4.5	11.0	90%	0.5	10.7	0.5
	8	3.6	18.1	1.3	8.5	2.3	10.2	93%	0.5		
	16	1.9	5.9	0.7	7.1	1.2	11.4	96%	0.6		
	32	1.0	3.5	0.3	13.9	0.7	10.3	110%	0.5		
IgG 2 20 mg/ml	4	8.9	12.1	4.3	1.3	4.6	17.0	92%	0.9	18.9	1.0
	8	4.6	13.0	2.4	12.0	2.1	19.4	85%	1.0		
	16	2.3	2.2	1.2	9.8	1.1	19.5	87%	1.0		
	32	1.2	12.3	0.6	11.6	0.5	19.7	86%	1.0		
IgG 3 60 mg/ml	12	5.0	3.0	0.3	4.6	4.7	3.7	95%	0.06	3.6	0.06
	24	2.3	6.2	0.1	8.8	2.1	3.5	85%	0.06		
	48	1.3	0.1	0.1	41.9	1.2	3.7	97%	0.06		
	96	0.7	7.2	0.04	21.8	0.6	3.6	103%	0.06		

13. Troubleshouting

Please visit the kit guidelines section on www.gyrosproteintechnologies.com/gyrolab-user-zone for more information and tips or contact your local field application specialist for support.

14. Disposal procedures

Gyrolab CDs and microplates shall be disposed of in accordance with federal, state and local environmental control regulations. The user is responsible for waste disposal and for providing suitable waste containers. Packaging material can be disposed through combustion for energy recovery.