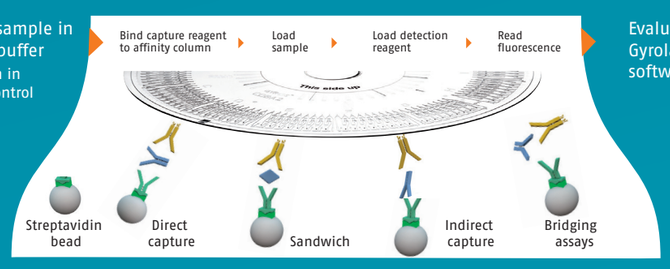
Gyrolab® immunoassay development guide

Automating nanoliter-scale immunoassays for more data in less time

1. Select the assay format

Prepare sample in Rexxip® buffer

Design run in Gyrolab Control software



Evaluate data in Gyrolab Evaluator software

Key steps automated in a Gyrolab Bioaffy™ CD and examples of formats.

Discover Gyrolab assay formats based on streptavidin surface chemistry

Sandwich assay: Format using two proteins which bind to different sites on the target analyte/ligand, referred to as capture and detection to form a sandwich. These proteins can be antibodies (Abs), peptides, or target receptors.

- **Direct capture:** a single biotinylated protein that binds to the target analyte/ligand
- **Indirect capture:** one biotinylated antibody and a secondary protein that binds to the target analyte/ligand to form the capture surface
- **Applications:** pharmacokinetics (PK), toxicokinetics (TK), biomarkers, affinity product titer and host cell protein (HCP) impurity immunoassays

Homogenous bridging assay:

- Anti-drug antibody (ADA) assay. For details on ADA assay development, see Gyrolab ADA assay protocol*

READY FOR VALIDATION

2. Identify reagent candidates

Focus on affinity, specificity and selectivity

Use affinity purified or antigen affinity purified polyclonal antibodies. These reagents are often a good starting point for a plate-based ELISA immunoassay. Orientation of capture or detection antibodies, or the antibodies themselves may need to be changed for optimal performance in Gyrolab platforms.

Consult reagent product data sheets

Data sheets often contain useful ELISA-based information, providing hints on which combinations to try, e.g. is the reagent a suitable capture or detection reagent, or suitable for different sample types? Are there recommendations on good antibody pairs?

Consider reagent formulation

Avoid protein additives (BSA, gelatin), amine buffers (Tris, glycine), and preservatives (Thimerosal, sodium azide), since these formulations affect labeling efficiency. Protein stabilizers need to be removed using affinity purification, and amine containing buffers require buffer exchange.

Consider all possible combinations

Depending on the drug species that is being measured (generic vs. specific) and the study approach (preclinical vs. clinical), consider all possible combinations for capture and detection.

Check for Gyrolab Assay Protocol

To accelerate assay development, start with carefully tested Gyrolab assay methods available for common applications including PK, COVID-19 antibodies, ADA, and biomarkers. The assays specify assay design, CD type, reagents, Rexxip buffer, dilutions, and needle wash buffers.

View Gyrolab Assay Protocols

3. Screen reagents

Label reagent candidates

Follow labelling protocols in Gyrolab User Guide.* Some reagent providers offer antibodies pre-conjugated with fluorophore or pre-biotinylated for additional convenience. To simplify reagent screening, start with Ab capture at 100 µg/mL and detect at 25 nM.

Parallel screening of multiple Ab pairs

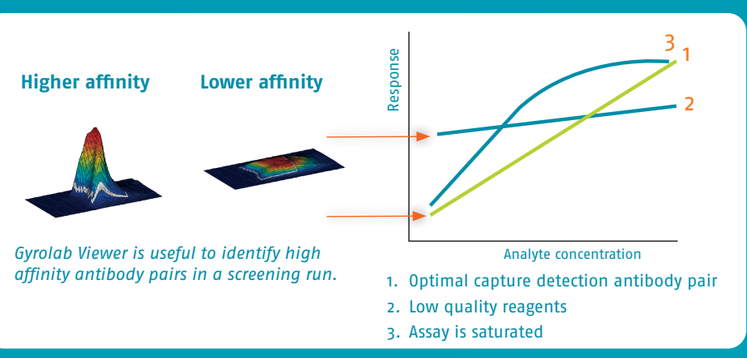
Using Gyrolab Bioaffy 200 CD, up to 7 Ab pairs can be screened using a seven point standard curve in singlicate (plus blank). Up to 14 Ab pairs can be screened using a three point standard curve in duplicate (plus blank).

Analyze data with Gyrolab Evaluator Software

- Compare standard curves of different candidates regarding signal to noise level and curve shape at high and low concentrations
- Blanks should ideally be low: instrument blank with PMT 1% typically gives a response of ~0.02 RFU
- The coefficient of variation (CV) for concentration determination should be low, and taken into account when comparing reagent combinations
- Poor precision may be caused by carry over between sample transfers: carry over is typically minimized by using the optimal Rexxip buffer and needle wash buffer, in combination with a two-wash Gyrolab method (see steps 6-7)

Take a closer look at on-column fluorescence with Gyrolab Viewer Software

Investigate outliers and compare binding profiles for best pair analysis.



Gyrolab Viewer is useful to identify high affinity antibody pairs in a screening run.

1. Optimal capture detection antibody pair
2. Low quality reagents
3. Assay is saturated

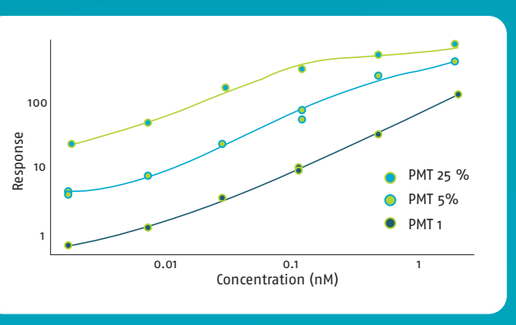
7. Optimize method

Discover Gyrolab Methods Database

A Gyrolab method determines how Gyrolab system processes samples and reagents. The User Zone on the Gyros Protein Technologies website (available to Gyrolab users) hosts a selection of Gyrolab Bioaffy methods in the Methods Database.* These include standard methods, and a range of additional methods that are useful when optimizing conditions for specific applications.

Improve dynamic range by analyzing different PMT settings

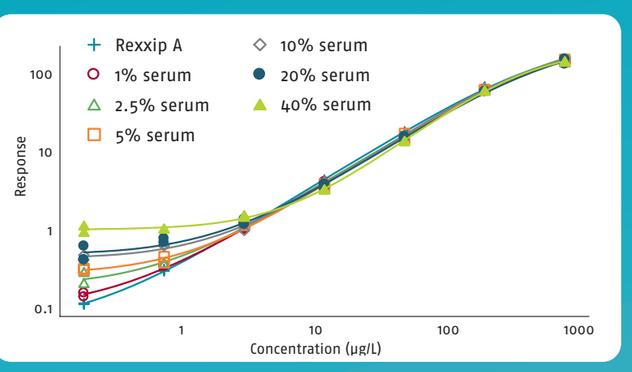
In the example below, the PMT settings of 5 and 25 % result in a plateau at the high end of the standard curve, which indicates saturation of the detector. A PMT-setting of 1% is in this case sufficient to cover the desired dynamic range.



- Optimize needle wash to improve precision
- If the analyte has a neutral isoelectric point (pI), and is not hydrophobic: Use a Gyrolab method that includes one needle wash step
- If the analyte has a pI≥8, or is hydrophobic, use a Gyrolab method that includes two needle wash steps
- Combine the two-wash method with a suitable Rexxip buffer
- Use Gyrolab Wash Buffer pH 11

Checking for carryover

When optimizing for the appropriate Rexxip buffer, run an 11 point standard curve (+ blank) in duplicate from high to low. Examine the low end for imprecision.



In the example above, MRD was determined by preparing standard curves in different concentrations of pooled serum (1-40%) diluted in Rexxip A. The optimal sample concentration was determined to be 5%.

*Protocols available on the Gyrolab User Zone at **www.gyrosproteintechnologies.com/gyrolab-user-zone**

SCAN ME

6. Assay optimization

Find the optimal Rexxip buffer

The Gyrolab platform affinity flow-through format eliminates incubations and minimizes matrix interference compared to ELISA. Start with a minimum required dilution (MRD) of 1:2.

Choose the dilution buffer from the range of Rexxip buffers, based on the analyte and sample type – See Rexxip selection guide.

Determine - MRD

Diluting the sample can improve accuracy, precision, and dynamic range. Start by measuring the analyte spiked into matrix at low, medium, and high levels. Then make a dilution series (e.g. 2-fold) with the selected Rexxip buffer. If the recovery is not in the range of 80–120 % (±20% bias), you may require further dilution to reduce matrix effects.

REXXIP BUFFER Additives* / Primary use	A / A-max	AN / AN-max SALT	H / H-max NEUTR	HN / HN-max SALT NEUTR	HX / HX-max NEUTR DET	F DET	CCS	ADA DET
Detection reagent buffer						X		
Recommended buffer if none of the below conditions apply	X							
Hydrophobic analyte					X	X		
Human and nonhuman primate samples (e.g. serum and plasma)								
Positively charged analyte, pI≥8				X				
Analyte with pI<8			X 1st option		X 2nd option			
Animal samples (nonprimate)								
Positively charged analyte, pI≥8		X						
Cell culture samples with high protein concentration Analyte is a therapeutic protein								
Positively charged analyte, pI≥8		X						
Analyte with pI<8							X	
Anti-drug antibody samples								
Acidic conditions								X

*All Rexxip buffers contain protective proteins and water based salt solution
SALT: Increased ionic strength
NEUTR: Reagents to neutralize heterophilic antibodies, e.g. HAMA
DET: Detergent
Rexxip-max buffers are designed for sample dilutions 1:2.
Regular Rexxip buffers should be used for sample dilutions > 1:2.

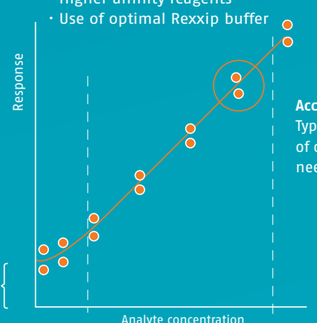
Rexxip selection guide

Aspects of a robust assay

Response level:
Typically increased by
- Higher affinity reagents
- Optimal detection reagent concentration
- Larger sample volume in CD (Gyrolab Bioaffy 1000)
- Higher PMT signal amplification

Background:
Caused by non-specific interaction between capture and detection reagents, and/or sample matrix. Affected by:
- Choice of reagents
- Rexxip buffer
- Sample dilution factor (MRD)

Sensitivity:
Typically improved by:
- Higher affinity reagents
- Use of optimal Rexxip buffer



Accuracy and precision:
Typically improved with use of optimal Rexxip buffer and needle wash method.

Lower end:
Determined by signal/background level.

Dynamic range:
Affected by reagent affinity and matrix effects, can be shifted by changing CD sample volume (e.g. Gyrolab Bioaffy 200 vs Gyrolab Bioaffy 20 HC), and PMT signal amplification.

Upper end:
Reflects the binding capacity, determined by the capture antibody affinity and concentration on column.

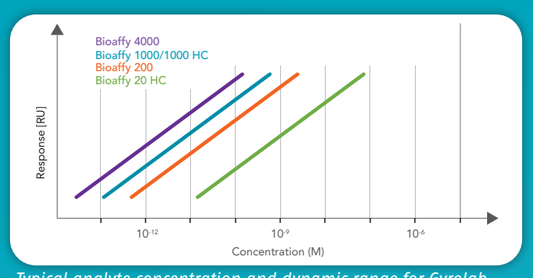
Learn more about Gyrolab Bioaffy CDs

4. CD selection

Choose CD depending on analytical need

Select the Gyrolab Bioaffy CD that accommodates the assay requirements for sensitivity and dynamic range. In general, assay sensitivity is greater with increasing CD sample volume capacity, or Bioaffy 20 < 200 < 1000 < 4000. Most assays can be run with Bioaffy 200. For highest sensitivity, try Bioaffy 1000 or Bioaffy 4000. For greater binding capacity, try Bioaffy 20 HC (eg, for IgG in cell culture samples) or Bioaffy 1000 HC (eg, for polyclonal or low affinity reagents) both contain porous HC particles.

For details on Gyrolab Mixing CD 96 and Gyrolab ADA assays, please refer to Gyrolab ADA Assay Protocols.*



Typical analyte concentration and dynamic range for Gyrolab Bioaffy CDs.

5. Titrate reagents

Capture reagent

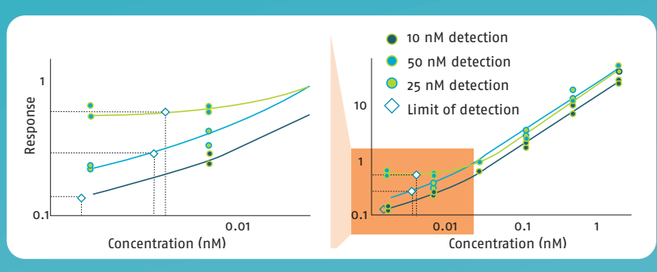
Sandwich assays: Saturate the affinity column with capture reagent, or 100 µg/ml (~700 nM) for Ab capture. Smaller capture molecules (<25 kDa) should start at ~2000 nM. Saturating the column eliminates the need to use a blocking reagent. Using significantly lower capture reagent concentrations may lower the column binding capacity, and also increase non-specific binding. In these situations an additional blocking step may be required.

Bridging assays: Saturation may cause binding of both arms of the drug antibody to the capture reagent. In these cases, titrate the capture reagent with biotinylated BSA.

Titrate the detection reagent

Start at 25 nm detection reagent and titrate down. Reducing the concentration of the detection antibody can improve performance by reducing background and increasing precision at low analyte concentrations. Examine the signal to background ratio as a criterion for optimal detection concentration.

In the example below, 10 nM detection reagent gives lower background and detection limit compared to 25 nM and 50 nM. The 10 nM detection reagent is still sufficiently concentrated to allow measurements at the high end of the standard curve, and is the recommended concentration.



For additional hints & tips visit **www.gyrosproteintechnologies.com**

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