PATsmart™ REBEL® System

Analyte Panel Performance Guide

26 Nov 2025

PATsmart™ REBEL® System Analysis—Calibration Range

SMA v2: Spent Media Analysis Kit, version 2

- As with all analytical assays, it is vital to ensure that analyte concentrations are within the appropriate dynamic range.
- Analyte concentrations below or above the calibration range will exhibit decreased reproducibility and accuracy.
- REBEL software has a built-in dilution guide— Range Finding Experiment Tool (RFE)—providing automated assessment of optimal dilution factors for the most inclusive analysis.

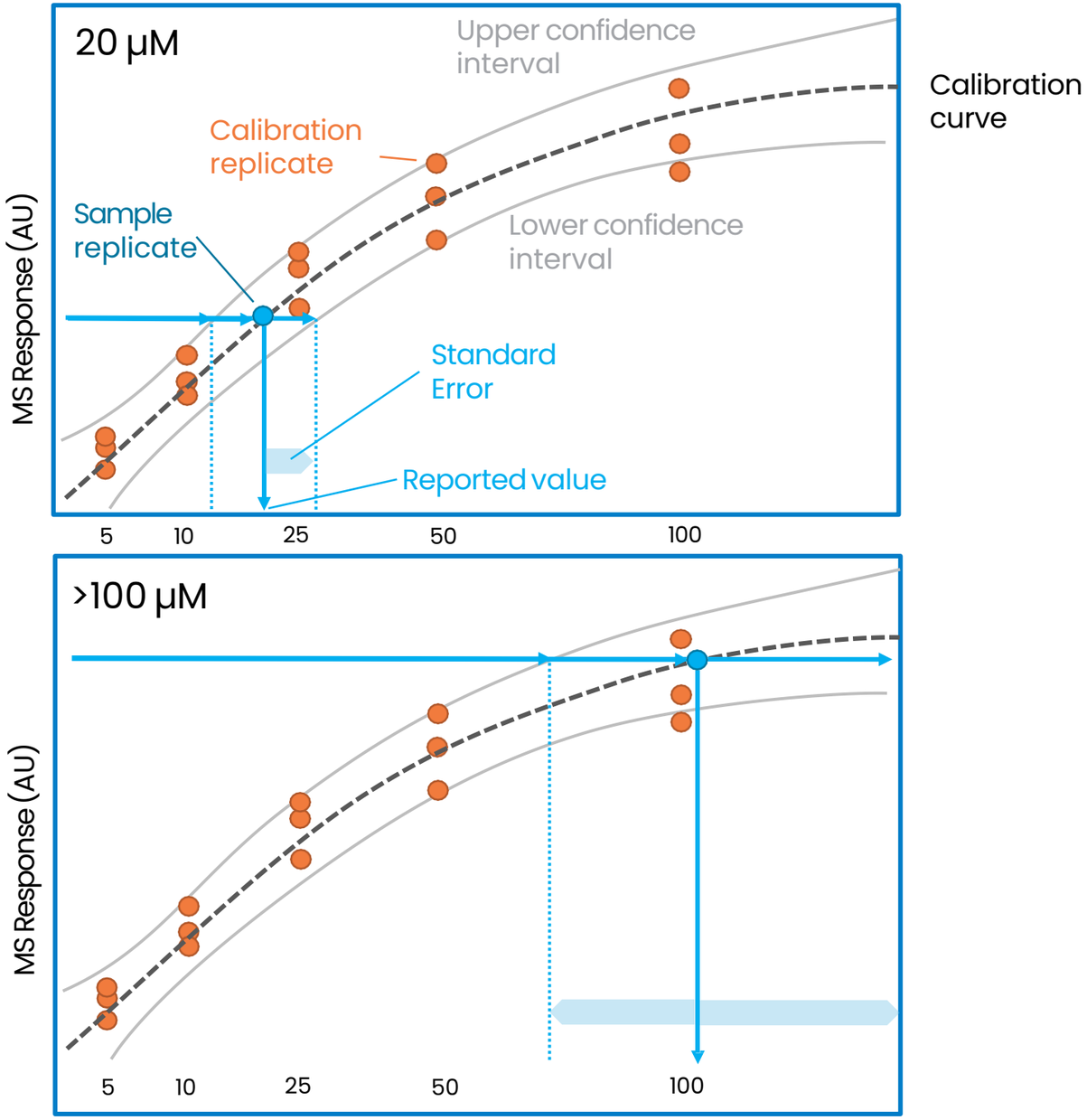
SMA V2 Analytes	Abbreviation	Lower Limit of Quantitation (LLOQ)	Upper Limit of Quantitation (ULOQ)
Alanyl-Glutamine	AQ	5 µM (0.005 mM)	100 µM (0.100 mM)
Alanine	Ala		
Arginine	Arg		
Asparagine	Asn		
Aspartic acid	Asp		
Betaine	Betaine		
Choline	Choline		
Citrulline	Cit		
Glycine	Gly		
Histidine	His		
Hydroxyproline	Hyp		
Isoleucine	Ile		
Leucine	Leu		
Lysine	Lys		
1-Methylhistidine	1MH		
Methionine	Met		
Phenylalanine	Phe		
Proline	Pro		
Serine	Ser		
Threonine	Thr		
Tryptophan	Trp		
Tyrosine	Tyr		
Valine	Val		
Glutamine	Gln	5 µM (0.005 mM)	75 µM (0.075 mM)
Glutamic acid	Glu		
Cystine	Cystine	5 µM (0.005 mM)	50 µM (0.050 mM)
β-Alanine	βAla	5 µM (0.005 mM)	25 µM (0.025 mM)
Thiamine	B1		
Pyridoxine	B6-OH		
Pyridoxal	B6-Oxo		
Nicotinamide	NAM		
β-Aminobutyric acid	GABA		
Sarcosine	Sarcosine		

REBEL Error Reporting

Understanding standard error on the REBEL

- REBEL reports all results in mM concentration accompanied by the associated standard error.
- Standard error and relative standard error (RSE) are statistical propagations of error for the system as a whole, based on the QC and individual sample replicate.
- Not to be confused with Coefficient of Variation (%CV) or Standard Deviation.
- The greater the RSE, the greater the uncertainty in the results .
    a) Most common cause for large RSE is extrapolation outside of the calibrated range, or at the extremes of the curve.
    b) Acceptable RSE are established by each user group, but generally <15% is considered good.

Relative standard error (RSE) can be interpreted as the expected variability in estimated concentrations over many replicates—samples are recommended to be run in triplicate.



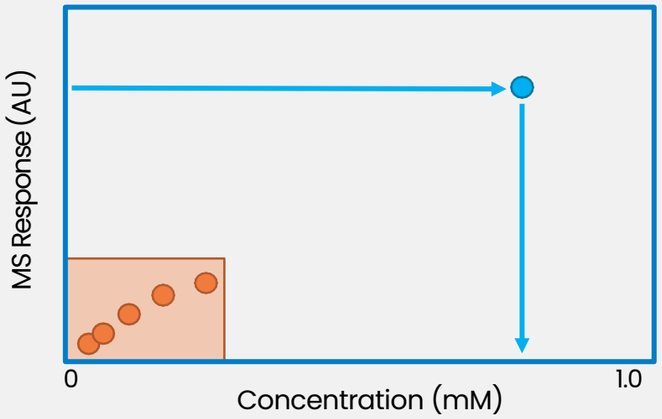
RSE for 20 µM concentration <<< RSE for >100 µM concentration

REBEL Analysis—Calibration Range

Determining whether analyte data is in/out of range

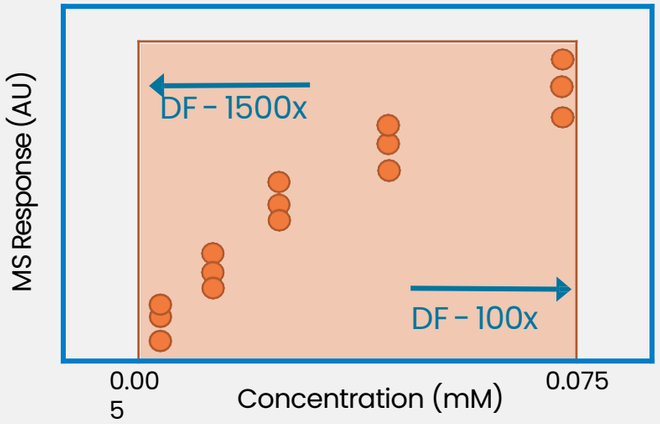
- When preparing samples, choosing the optimal dilution factor prior to analysis is vital to ensuring precise and accurate results. Range Finding Experiment (RFE) tool helps optimize dilution factor for unknown samples.
- The proper dilution should bring samples in range so results fall within the standard calibration curve post-dilution or risk lower confidence/higher relative standard error (RSE) in resulting data.
- All samples require a minimum of at least 10x dilution in REBEL diluent.

Example: Gln at 7.5 mM concentration



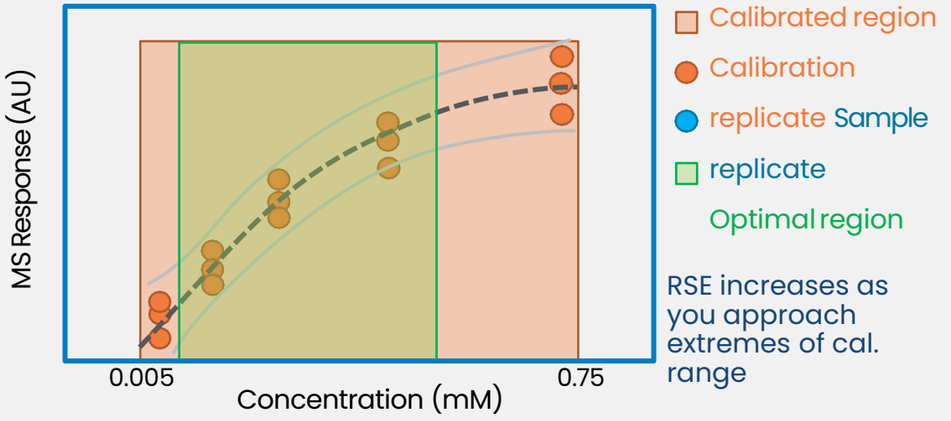
(**a**) Cal. range for Gln = 0.005 mM–0.075 mM. To be in range, the sample must be dilute enough to get below 0.075 mM but not so much as to fall below 0.005 mM.

Minimum 10x dilution means sample concentration will fall well above cal. range at 0.75 mM, resulting in high RSE and low confidence in accuracy, among other concerns.



(**b**) Lower/upper limits are:
7.5 mM / 0.005 mM = 1500x;
7.5 mM / 0.075 mM = 100x.

To be in range, the sample must be diluted at least 100x but no more than 1500x.



(**c**) Being in calibration range is vital, while getting within optimal range is ideal—optimal range is an approximate, tighter window within the calibrated range that affords more robust data with lower RSE—individual to each analyte and is represented by the thicker line on the Dilution Report chart generated from running an RFE with the tool provided on the REBEL.

Analytes frequently in low levels in cell culture media

REBEL analyte panel considerations

- Vitamins and other amines in the analyte panel may present challenges based on their conventionally lower concentrations in most media, and the smaller dynamic range within which the analytes are calibrated for analysis.
- Further optimization of dilution factor distinct from AAs may be required—i.e., a lower dilution factor is often necessary to accurately quantify these analytes.

Analyte full name	Abbreviation
Vitamins	
Choline	Choline
Pyridoxine	B6-OH
Pyridoxal	B6-Oxo
Nicotinamide	NAM
Thiamine	B1
Amines	
γ-aminobutyric acid	GABA
1-Methylhistidine*	1MH
Citrulline	Cit
Hydroxyproline	Hyp
Sarcosine	Sarcosine

*1-methylhistidine cannot be distinguished from 3-methylhistidine

Analytes with specific considerations in cell culture media

REBEL analyte panel considerations

- For optimal performance of all analytes, please use standards and reagents within expiration.
  - > Posted Expiration—BGE/Diluent expire 1 year after manufacture; Standards expire 3 months after manufacture.
  - > Use Expiration—BGE/Diluent expire 30 days after opening; Standards expire 14 days after initial use.

Analyte full name	Abbreviation	Comment
β-Alanine	βAla	Measurements may be less precise
Arginine	Arg	Measurements may be less precise
Cystine	Cystine	May be prone to degradation
Glutamine	Gln	Possible co-migration with Glu
Glutamic acid	Glu	Possible co-migration with Gln
Glycine	Gly	Measurements may be less precise
Serine	Ser	Measurements may be less precise
Valine	Val	Possible co-migration with Sarcosine

- "Measurements may be less precise"—under certain conditions or in some media, the measurements resulting for this analyte may be less reproducible than others.
- "May be prone to degradation"—analyte is known to be more labile under certain conditions.
- "Possible co-migration"—analyte known to encounter detection interferences with other analytes, especially at higher concentrations.

Summary

REBEL performance is expected to be accurate and precise, while there are notable analytes that may prove more challenging based on observations, this information is good to keep in mind when analyzing REBEL data or preparing dilutions.

Routine Amino Acids

Analyte full name	Abbreviation
Alanyl-Glutamine	AQ
Alanine	Ala
Asparagine	Asn
Aspartic acid	Asp
Glutamine + Glutamic Acid	Glx
Histidine	His
Isoleucine	Ile
Leucine	Leu
Lysine	Lys
Methionine	Met
Phenylalanine	Phe
Proline	Pro
Threonine	Thr
Tryptophan	Trp
Tyrosine	Tyr

Vitamins + Amines[1]

Analyte full name	Abbreviation
Vitamins	
Choline	Choline
Pyridoxine	B6-OH
Pyridoxal	B6-Oxo
Nicotinamide	NAM
Thiamine	B1
Amines	
γ-aminobutyric acid	GABA
1-Methylhistidine[2]	1MH
Citrulline	Cit
Hydroxyproline	Hyp
Sarcosine	Sarcosine

[1] These analytes may present challenges based on their lower concentrations in media and smaller dynamic range for analysis.
[2] 1-methylhistidine cannot be distinguished from 3-methylhistidine.

Analytes with specific considerations

Analyte full name	Abbreviation	Comment
β-Alanine	βAla	Measurements may be less precise
Arginine	Arg	Measurements may be less precise
Cystine	Cystine	May be prone to degradation
Glutamine	Gln	Possible co-migration with Glu
Glutamic acid	Glu	Possible co-migration with Gln
Glycine	Gly	Measurements may be less precise
Serine	Ser	Measurements may be less precise
Valine	Val	Possible co-migration with Sarcosine

Expirations and LOQ

Reagent	Expiration
Standards	14 days after initial use (or 3 months from manufacture – posted date)
BGE / Diluent	30 days after opening (or 1 year from manufacture – posted date)

LLOQ/ULOQ (mM)	Analytes
0.005 / 0.025	βAla, B1, B6-OH, B6-Oxo, NAM, GABA, Sarcosine
0.005 / 0.050	Cystine
0.005 / 0.075	Gln, Glu
0.005 / 0.100	All other analytes

We’re here to help!

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Call us 24/7/365

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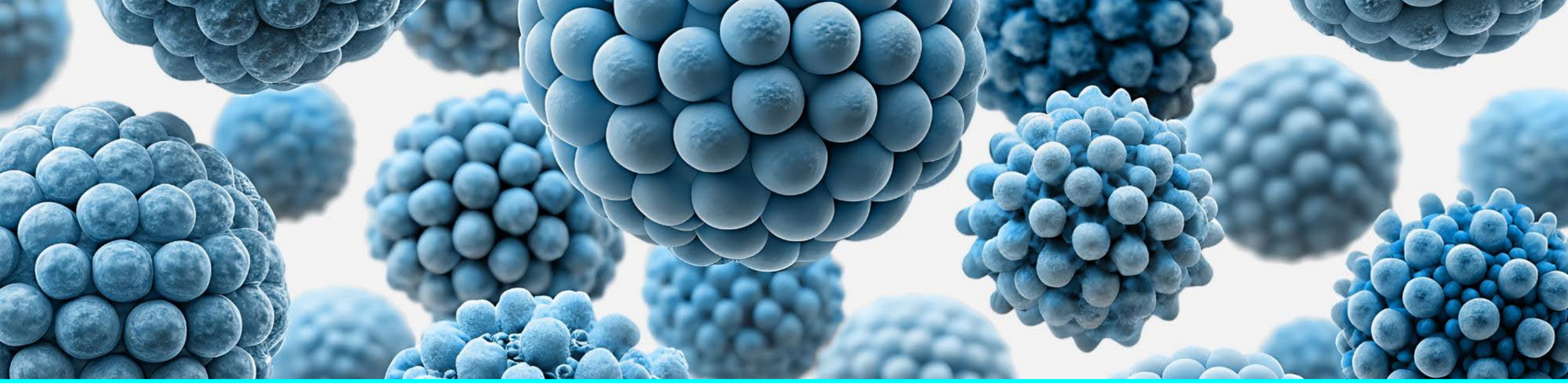
www.repligen.com/techsupport

For further questions and best practices, please contact your Repligen field applications scientist.



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