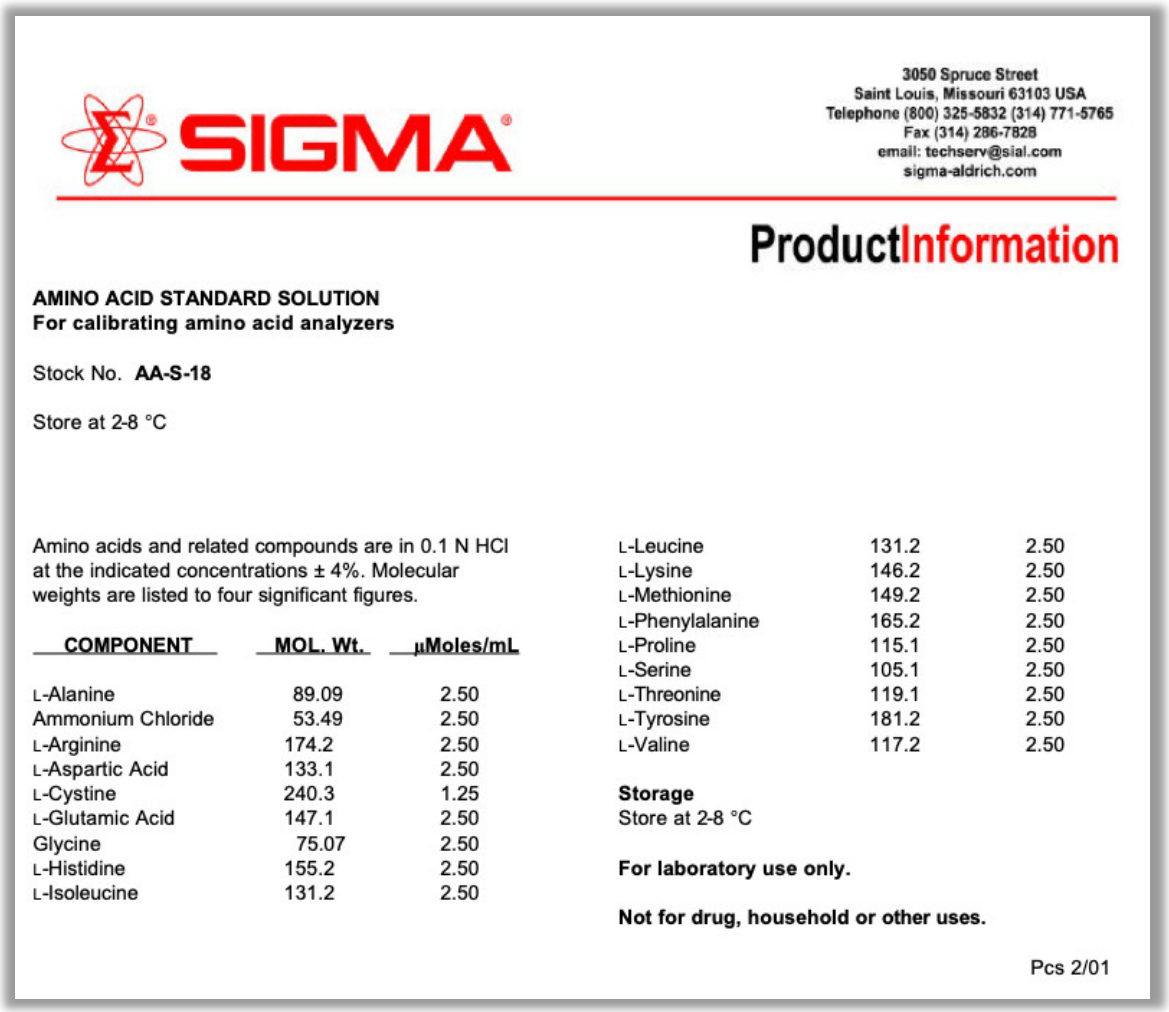
REBEL Validation Protocol

Introduction

The procedures listed in this document are intended to serve as a guide for in-house validation of the PATsmart™ REBEL® Cell Culture Media Analyzer. The third-party standard recommended in this document is Millipore Sigma AAS18 Amino Acid Standard Solution. The details of this solution are shown below.



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ProductInformation

AMINO ACID STANDARD SOLUTION
For calibrating amino acid analyzers

Stock No. **AA-S-18**

Store at 2-8 °C

Amino acids and related compounds are in 0.1 N HCl at the indicated concentrations ± 4%. Molecular weights are listed to four significant figures.

COMPONENT	MOL. Wt.	µMoles/mL
L-Alanine	89.09	2.50
Ammonium Chloride	53.49	2.50
L-Arginine	174.2	2.50
L-Aspartic Acid	133.1	2.50
L-Cystine	240.3	1.25
L-Glutamic Acid	147.1	2.50
Glycine	75.07	2.50
L-Histidine	155.2	2.50
L-Isoleucine	131.2	2.50
L-Leucine	131.2	2.50
L-Lysine	146.2	2.50
L-Methionine	149.2	2.50
L-Phenylalanine	165.2	2.50
L-Proline	115.1	2.50
L-Serine	105.1	2.50
L-Threonine	119.1	2.50
L-Tyrosine	181.2	2.50
L-Valine	117.2	2.50

Storage
Store at 2-8 °C

For laboratory use only.

Not for drug, household or other uses.

Pcs 2/01

Of the 18 components listed, 17 are relevant to REBEL: *Ala, Arg, Asp, Cys, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Tyr, and Val*. Note that the Millipore Sigma AAS18 standard solution contains 16 amino acids at a concentration of 2.5 mM ±4%, and one amino acid (*Cys*) at a concentration of 1.25 mM ±4%.

Materials Needed

- REBEL SMA Kit
- Millipore Sigma AAS18 Amino Acid Standard (5mL)
- Calibrated pipettes
  - 100 µL
  - 1000 µL
  - 5 mL
- HPLC Grade Water
  (or equivalent, i.e., RO/DI water)
- Vortex Shaker (optional)

Pipetting Accuracy Study

- Calibrated microbalance
- Weigh boats

Validation Study

- Five 10-mL (or larger) centrifuge tubes
- Ten 2-mL autosampler vials and caps (example)

Pipetting Evaluation

It is strongly recommended to assess the accuracy and precision of pipetting to support the total analytical error analysis.

1. Using a 100 µL calibrated pipette, place 50 µL of HPLC Grade (or RO/DI) water in a tared weighing boat on a calibrated microbalance and record the reported mass.
2. Repeat this step nine additional times. You may fill in the table below for convenience.

Measurement	Mass (mg)
1	
2	
3	
4	
5	
6	
7	
8	
9	
10	

3. Calculate the average (mean) value, standard deviation, and relative standard deviation of the mass measurements:

   AVERAGE VALUE (AVG): _____

   STANDARD DEV (STD): _____

   %RSD = 100 x STD/AVG _____

4. Compare your *AVERAGE* value to the expected mass of water given your laboratory temperature. If your laboratory environment is within five degrees of 20°C, you can safely assume a density of 0.998 g/mL for water, and therefore a "true" mass of 49.9 mg for a 50 µL volume.

Validation Procedure

REBEL Setup and Initialization

1. Ensure REBEL is running the latest software version.
2. Power on REBEL.
3. Empty the Waste Bottle.
4. Install a new REBEL chip, BGE, and standards as recommended in the User Manual.
5. Run a Self-Test (*Settings* > *Tools*).
6. Run a Quantitative Calibration (*Settings* > *Tools*).
7. If any alerts appear during Self-Test or Quantitative Calibration, repeat the procedure. If the problem persists, contact your Field Application Scientist or Repligen help line at TechSupport@repligen.com.

Stock Sample Preparation

8. Prepare five centrifuge tubes and label them consecutively “1” through “5”.
9. Using a 100 µL calibrated pipette, pipette 50 µL of AAS18 standard solution into each centrifuge tube.
10. Using a 5-mL calibrated pipette, pipette 4950 µL of REBEL Diluent into each centrifuge tube.
11. Mix each stock solution tube well (vortex shaker recommended).

Sample Vial Preparation

12. Prepare ten autosampler vials
    a. Label the vials in five pairs (“1-1”, “1-2”, …, “5-1”, “5-2”).
    b. From Stock Sample tube #1:
        I. aliquot 1000 µL to the vial labeled “1-1”
        II. aliquot 1000 µL to the vial labeled “1-2”.
    c. Repeat for each Stock Sample and its corresponding vials.
13. Import the REBEL Validation Study Batch Runsheet, which will be provided by your Field Application Scientist (*Plus icon* [*“+”*] > *Import Runsheet* > navigate to REBEL Validation Study Runsheet).
14. Select *Add Batch to Queue* and confirm the non-selected check boxes.
15. Select *Start*.

Validation Data Analysis

1. Export results files from REBEL. There should be 40 run results within the "…-results.csv" file if the protocol was performed using the recommended number of stock solutions, vials, and replicates noted above.
2. Reduce the data reported to the 17 amino acids relevant to REBEL:
   Ala, Arg, Asp, Cys, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Tyr, and *Val.*
3. Arrange the data in tabular form, as shown below. (If using the Validation Excel Workspace file provided by your Field Applications Scientist, simply copy & paste your results into the file).

Control Media (Example)						
Analyte	**Sample 1, rep 1 (mM)**	**Sample 1, rep 2 (mM)**	**(…)**	**Sample 10, rep 4 (mM)**	**Average* (mM)**	**Standard Deviation****
Alanine	2.32	2.71	…	…	…	…
Arginine	2.55	2.45	…	…	…	…
Asparagine	2.71	2.35	…	…	…	…
Cystine	0.99	1.52	…	…	…	…
Glutamic Acid	2.02	2.68	…	…	…	…
…	…	…	…	…	…	…
Val	2.21	2.81	…	…	…	…

4. Create a new table for %recovery values, where for each cell, the reported value is divided by the estimated true concentration listed on the stock sample label—the % recovery. If any values are reported with Relative Standard Errors > 30%, discard the values and leave them blank in the summary table.

Relative Results (Example)			
Analyte	%Recovery=100*(measured result/label) Sample 1, rep 1 (mM)	…	Average %Recovery (mM)
Alanine	100*(2.32/2.5)	…	…
Arginine	100*(2.55/2.5)	…	…
Asparagine	100*(2.71/2.5)	…	…
Cystine* **	100*(0.99/**1.25**)	…	…
Glutamic Acid	100*(2.02/2.5)	…	…
…	…	…	…
Val	100*(2.21/2.5)	…	…

**To calculate average in Excel, enter the formula "`=average(Cell Range)`". Be sure to calculate 11 averages for each analyte: one for total average across all replicates, one for Sample average across each prepared vial.*

***To calculate Standard Deviation in Excel, enter the formula:"`=STDEV(Cell Range)`". Be sure to calculate 11 standard deviations for each analyte: One for total Standard Deviation across all replicates, one for Sample Standard Deviation across each prepared vial.*

****The concentration for **cystine** in the AAS18 standard is **half** the concentration of the other analytes.*

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Total Method Error Summary

The total analytical error from this validation study is the combination of all sources of error from throughout this procedure. Ultimately, REBEL error cannot eliminate the error inherent in standard solution or introduced during the preparation process.

Summarize the values obtained from the evaluation in the following table for each analyte and calculate accuracy and precision for REBEL as shown in the last row.

Contributor—Reference	**Observed / Known**
Standard solution spec	+/- 4% (from standards manufacturer)
Pipette accuracy* record	+/- AVG from pipette study, or assume +/- 5%
Pipette precision* record	+/- STD from pipette study, or assume +/- 5%
Reference total error	= (4%2 + ACC2 + 2*PREC2)1/2 accuracy and precision values from pipette study
Contributor—REBEL Observed	
REBEL Assay accuracy	Average recorded value across all analytes and runs
REBEL Assay precision	Standard deviation across all analytes/runs. Precision of the REBEL assay can be improved by combining (averaging) several replicates together. Generally, the precision is expected to improve with the square root of the number of replicates. For example, four replicates will improve the precision by approximately 50%.

**Pipette accuracy and precision are often operator dependent and can be 20% or more in some cases. Many pipette companies such as Eppendorf offer free training on proper pipetting techniques to reduce this source of error.*

Example	
Contributor—Reference	**Measured/calculated values**
Standard solution spec	+/- 4%
Pipette accuracy record	4.5%
Pipette precision record	7.3%
Reference total error	= 11.9%
Contributor—REBEL Observed	
REBEL Assay accuracy	5.6%
REBEL Assay precision	15.8% (individual replicates) 9.5% (4 replicate averages)

A=accuracy of the analyte, calculated from the average error across all replicants per analyte P=precision, calculated from the average %RSD across all precision per analyte

REBEL Accuracy (for each analyte):**

$$A \pm 1.96 * \left(\frac{P}{6.24}\right)$$

REBEL Precision (for each analyte):**

Upper bound = $\left(39 * \frac{P^2}{58.12}\right)^{\frac{1}{2}}$

Lower bound = $\left(39 * \frac{P^2}{23.65}\right)^{\frac{1}{2}}$

***Critical values for t- and chi-squared with 40 degrees of freedom*

For 24/7/365 Customer Support

Please contact TechSupport@repligen.com for further information about our reagent kits.