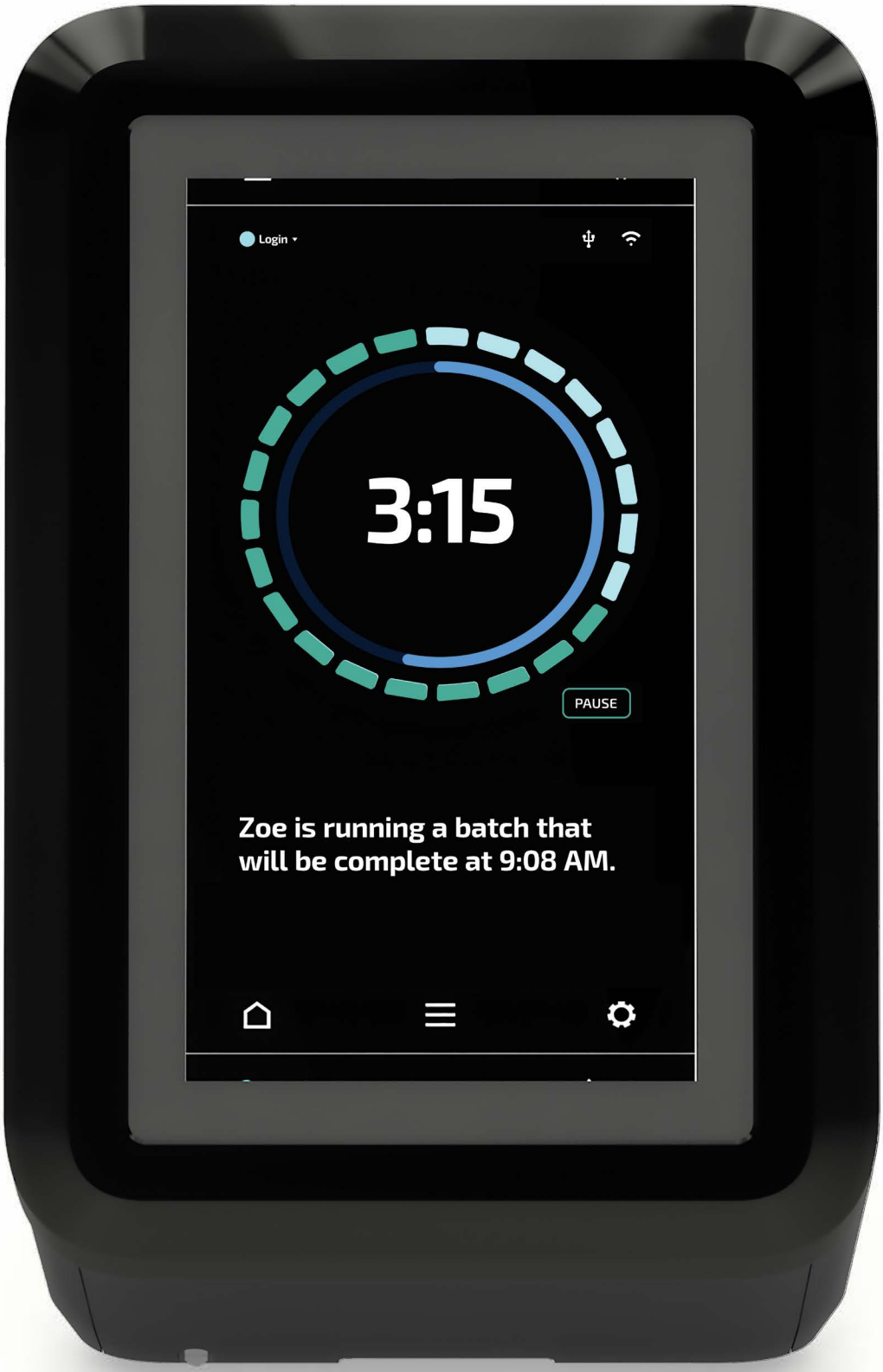

REBEL

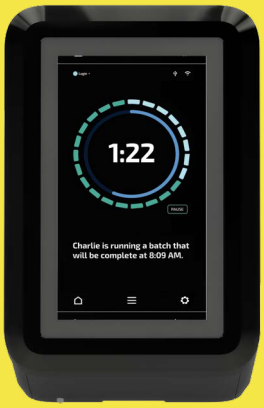

Login
3:15
PAUSE
Zoe is running a batch that
will be complete at 9:08 AM.

CELL AND GENE THERAPY

REBEL

APPLICATION SNAPSHOT

Comparison of various conventional and chemically-defined T cell medias.



BACKGROUND

T cells are lymphocytes that have special cell surface receptors that recognize antigens, which is vital for an immune response. Recently, chimeric antigen receptor (CAR) T cells have been created to target particular antigens on tumor cells as a type of immunotherapy. CAR-T cell therapies are currently involved in numerous clinical trials as a potential breakthrough therapy across a wide variety of malignancies. CAR-T cells are commonly grown and expanded in RPMI (Roswell Park Memorial Institute) 1640 basal media supplemented with up to 10% fetal bovine serum (FBS). For clinical applications, there is a desire to use cell media platforms that allow for higher expansion rates, have more lot-to-lot consistency between batches, and exclude FBS and other animal-derived components due to concerns over contamination. Chemically-defined media provides improved batch-to-batch results and removes some regulatory concerns since it excludes serum products. However, serum-free media may yield lower CAR-T efficacies, so understanding how to optimize the media to improve the therapy is a necessary step.

THE EXPERIMENT

In this study, the Rebel was used to probe the essential amino acid content of RPMI + FBS, combo (both base and expansion) media with and without human serum albumin (HSA), base media alone, and expansion media alone. All media samples were handled following the manufacturers' instructions. The samples were diluted 25x before analysis on the Rebel with no additional sample preparation. Each media sample was run five times. (Figure 1)

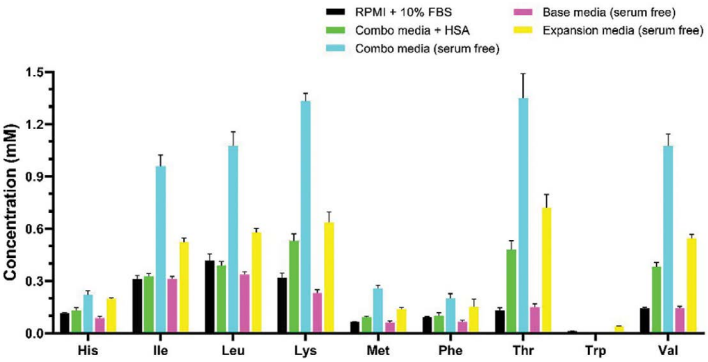


Figure 1: The concentrations of the essential amino acids in five cell media platforms used for T-cell culturing. Error bars are from the standard deviation of n = 5 replicates.

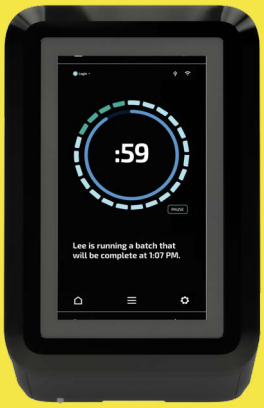
DISCUSSION

There were significant differences in the essential amino acid content between the traditional RPMI with 10% FBS media and the chemically-defined media for T cell growth. The serum-free combination media (used as both a base and expansion media, blue) had the highest levels of most essential amino acids. The serum-free expansion media (yellow) had the second-highest levels of all the media and had the highest levels of Trp. In fact, other than the serum-free expansion media, only the RPMI + 10% media had Trp detected in the formulations. RPMI + 10 % serum media (black) and the serum-free chemically-defined base media (pink) had the lowest levels of the essential amino acids. A combination of media that included HSA (green) had essential amino acid levels that were moderate when compared to the highest and lowest media. With this quick insight from the Rebel, researchers can make informed decisions about which media platform is best for growing CAR T-cells.

REBEL

APPLICATION SNAPSHOT

Comparing mesenchymal stem cell media with supplements on the Rebel.



BACKGROUND

Mesenchymal stem cells (MSCs) are multipotent adult stem cells, which can differentiate into specialized cell types such as bone (osteoblasts), cartilage (chondrocytes), and fat (adipocytes). In addition to differentiation, MSCs have the capacity for self-renewal, so their therapeutic potential is a topic of high interest for tissue engineering and regenerative medicine. In the lab, cultures of MSCs are fed cell media that has been specifically designed to maximize the MSC growth potential, and the media commonly includes serum. However, due to the variability in serum compositions and the risk associated with animal component-containing media, many research groups have adopted serum-free culturing practices to grow MSCs. This practice allows groups to have more consistency between experiments while meeting regulatory requirements for clinical studies. In the absence of serum, proprietary supplements are added to the MSC cultures that provide growth factors for both expansion and differentiation.

THE EXPERIMENT

Two different commercial serum-free MSC media without glutamine were tested. The MSC media was tested both with and without the supplied supplements to evaluate the change in the amino acid profile between the two formulations. All media samples were prepared following the manufacturers' protocols and diluted 10x before analysis on the Rebel™ with no additional sample preparation. (Figure 1)

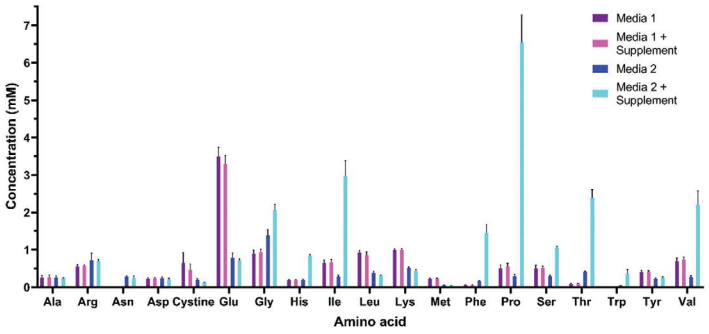


Figure 1: Media amino acid profile from chemically-defined MSC media with and without the supplied supplements. Error bars are from the standard deviation of n = 5 replicates.

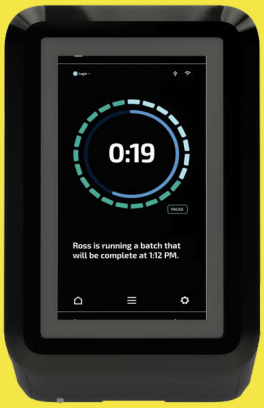
DISCUSSION

There was no detected difference in the amino acid levels of Media 1 when the supplement was added, indicating that the supplement did not contain additional amino acids in the formulation. However, in Media 2, there was a significant increase in the concentration of nine amino acids - Gly, His, Ile, Phe, Pro, Ser, Thr, Trp, and Val. Of these amino acids, Gly showed the lowest increase after the supplement was added (1.5x increase). On the other end, Ile (10.4x higher) and Pro (22.4x higher) both had the most substantial increases in concentration after the supplement was added. The other six amino acids increased their levels anywhere between 3x - 8.5x with the supplement added compared to the base media alone. To ensure consistency when culturing MSCs, researchers should understand what is being added to their media. The Rebel is a simple tool to analyze MSC media as it is freshly prepared and during the culturing process to probe the nutrient uptakes of their cells.

REBEL

APPLICATION SNAPSHOT

Comparison of the essential amino acid contents of Sf9 and Sf21 insect cell media.



BACKGROUND

Insect cell lines like Sf9 and Sf21 were isolated initially from *Spodoptera frugiperda*. They are commonly used for recombinant protein expression, vaccine development, and viral vector production. These cell lines are very adaptable to both culturing in adherent and suspension cultures, and in traditional media with serum, serum-free media, and protein-free media. The selection of the appropriate media is based on a variety of conditions, but one of the most important is the amino acid content. Many amino acids are not synthesized by insect cells, and some are crucial to be added to cell media for optimal growth and/or protein production. Knowledge of the insect cell metabolism coupled with an understanding of the amino acid content of the cell media allows for a more informed process development analytics strategy to correlate changes in cell growth/viability and productivity to the nutrient contents of the media.

THE EXPERIMENT

Three different cell medias intended for Sf9 or Sf21 insect cell line growth were used in this study. The insect cell media tested included a traditional media, a chemically-defined media, and a serum-free media. All media samples were handled following the manufacturers' instructions. The samples were diluted 100x before analysis on the Rebel with no additional sample preparation. For simplicity, only the essential amino acids are shown for a comparison of the three media. (Figure 1)

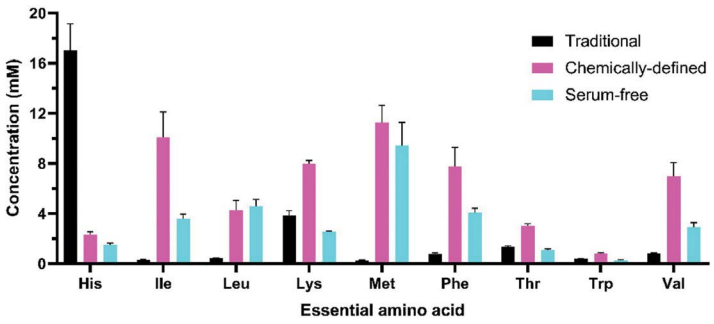


Figure 1: Essential amino acid profile from traditional, chemically-defined, and serum-free insect media. Error bars are from the standard deviation of n = 5 replicates.

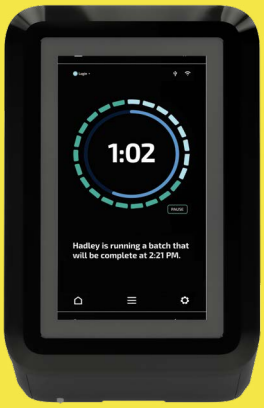
DISCUSSION

There were varying levels of all the essential amino acids, regardless of the type of media formulation. The traditional media had substantially higher levels of His compared to the chemically-defined and serum-free media by 7.4x and 11.3x, respectively. However, the traditional media had the lowest levels of five amino acids – Ile, Leu, Met, Phe, and Val. The chemically-defined media had the highest levels of Ile, Lys, Met, Phe, Trp, and Val. Also, it was tied with the serum-free media for the highest levels of Leu. Other than Leu, the serum-free media had the second or third most amount of any other essential amino acid shown here. This brief analysis demonstrates how it is vital to consider measuring the composition of amino acids in insect cell media. Researchers should consider how the amino acid composition, in conjunction with the undefined components in traditional media (e.g., serum) and serum-free media (e.g., peptones, hydrolysates, yeastolates), may all affect the growth and productivity of their processes.

REBEL

APPLICATION SNAPSHOT

Supplementing RPMI media with different amounts of fetal bovine serum.



BACKGROUND

Roswell Park Memorial Institute (RPMI) developed RPMI-1640 media from modification to McCoy's 5A media. RPMI media is one of the most used chemically-defined and protein-free cell media since it works across a variety of mammalian cell lines (e.g., stem cells, T and B cells, hybridomas, HEK293, THP-1 leukemia cells, etc.) grown in suspensions and as monolayer cultures. RPMI media is usually supplemented with fetal bovine serum (FBS) at levels of 1 to 10% to supplement growth factors necessary to sustain specific cell line processes. However, FBS supplemented media presents some issues when there is a desire to perform routine quantitative analysis of the media. The high serum protein contents in the mixed media may interfere with standard chromatography columns for amino acid analysis. Removal of the proteins with precipitation may be required to prepare the samples before analysis with traditional chromatographic platforms. This additional sample prep may affect amino acid recoveries resulting in biased results with conventional analytics approaches for media analysis.

THE EXPERIMENT

A commercially available RPMI media (without glutamine) and cell culture grade FBS (USA origin) were mixed at the listed levels below. All media samples were handled following the manufacturer's instructions. Final solutions were diluted 10x before analysis on the Rebel with no additional sample preparation. (Figure 1)

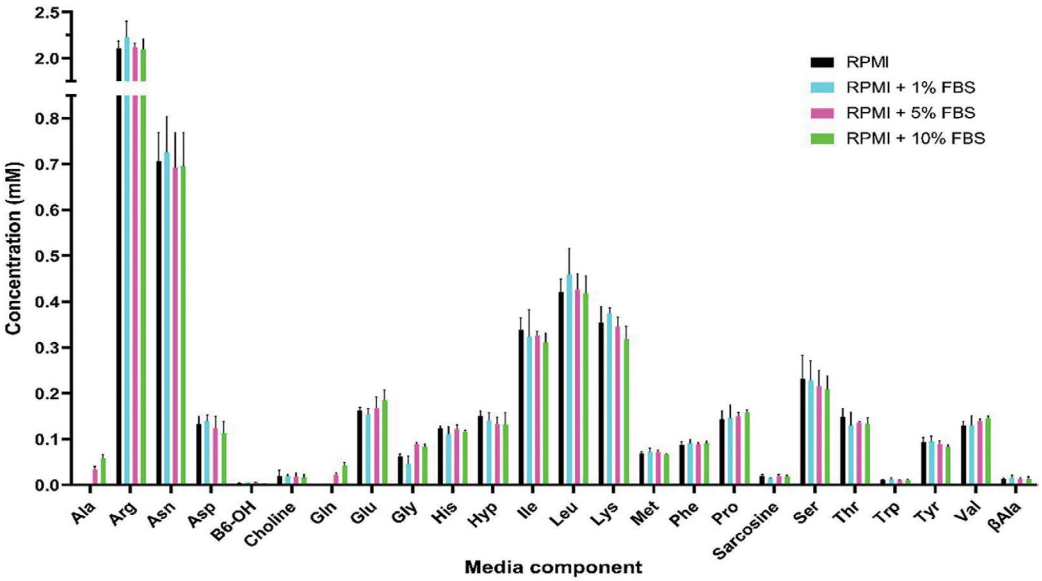


Figure 1: Media component concentrations from RPMI and RPMI with FBS supplementation. Error bars are from the standard deviation of n = 6 replicates.

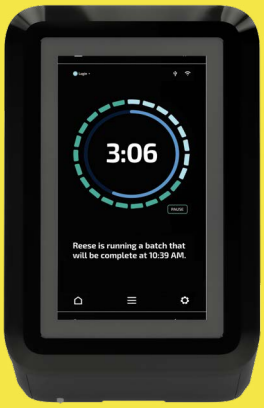
DISCUSSION

The analysis was able to quantitate 24 different media components, including 19 amino acids, at a single dilution level. There were virtually no differences in the media components' concentrations between the base RPMI media and the RPMI samples with up to 10% FBS added. The exceptions to this observation were both Ala and Gln. Both Ala and Gln were not in the original formulation of RPMI. They were not detected in the base media and 1% FBS-supplemented media. However, in 5% and 10% FBS, both Ala and Gln were identified, which were consistent with the separate observation of detecting both these amino acids in pure serum tested at a 10x dilution (not shown). Corresponding to the formulation, Arg had the highest levels of all amino acids averaging 2.108 mM. In comparison, Trp had the lowest concentration with an average of 0.011 mM. With the Rebel, one can quickly and confidently screen serum-supplemented media formulations to ensure batch-to-batch media consistency before introducing the cell media to cultures.

REBEL

APPLICATION SNAPSHOT

Supplementing IMDM media with different amounts of fetal bovine serum.



BACKGROUND

Iscove's Modified Dulbecco's Medium (IMDM) is an amino acid, vitamin, and inorganic salt enriched version of Dulbecco's Modified Eagle Medium (DMEM). IMDM is commonly used for high-density and high-proliferating cell cultures of fibroblast-like cell lines (e.g., COS-7), hematopoietic stem cells, macrophages, and T lymphocytes (e.g., Jurkat cells). Since IMDM is a chemically-defined media, it lacks growth factors, proteins, and lipids. To support cell growth, IMDM is often supplemented with fetal bovine serum (FBS) at various levels (e.g., 1 to 10%) depending on the cell line. When using chemically-defined cell media like IMDM supplemented with FBS, media analysis can be burdensome. Interferences arise between the high levels of the proteins in the added FBS with standard chromatography columns. Additional sample preparation may be required to remove and precipitate the proteins from the sample media. These added steps may affect recoveries of the trace media components (e.g., amino acids and vitamins), resulting in issues with conventional analytics approaches for media analysis.

THE EXPERIMENT

A commercially available IMDM media without glutamine and cell culture grade FBS (USA origin) were mixed at the listed levels below. All media samples were handled following the manufacturer's instructions. Final solutions were diluted 20x before analysis on the Rebel with no additional sample preparation. (Figure 1)

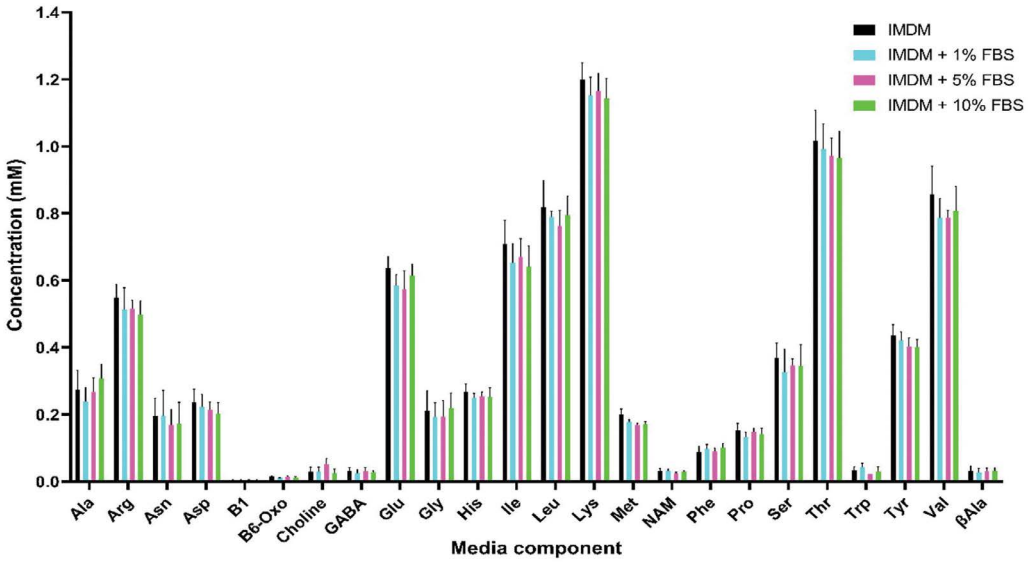


Figure 1: Media component concentrations from IMDM and IMDM with FBS supplementation. Error bars are from the standard deviation of n = 6 replicates.

DISCUSSION

At a single dilution of 20x with Rebel diluent, the analysis was able to quantitate 24 different media components, including 18 amino acids and three B vitamins. There were virtually no differences in the media components' concentrations between the base IMDM media alone and the IMDM samples with up to 10% FBS added. Amongst the amino acids, Trp had the lowest levels averaging 0.032 mM, and lysine had the highest average concentration of 1.165 mM across the four formulations tested. Vitamin B1 (thiamine) was the lowest concentration of all the media components detected with an average concentration of 0.004 mM. Gamma-aminobutyric acid (GABA) and beta-alanine (βAla), which are not universally present in all chemically-defined media formulations, were detected across all samples at average concentrations of 0.028 mM and 0.030 mM, respectively. With the Rebel, one can quickly and confidently screen serum-supplemented media formulations. This test ensures batch-to-batch supplemented media consistency before introducing the media to cell cultures.

SPECIFICATIONS

REBEL

SPEC SHEET

Get cell media analysis at-line, anytime.

The Rebel puts the control in your hands.

Its small, self-contained footprint fits right on your bench allowing you to run samples at-line in less than 7 minutes.

REBEL

SPECIFICATIONS

SAMPLE INFO

Get quantitative identification and analysis on a panel of >30 analytes including all the key amino acids, biogenic amines, water soluble vitamins and dipeptides.

Analysis time:
7 minutes per well

Volume and format:
10 µL minimum volume (prior to dilution)
96-well plate or chromatography vials
Cooled sample storage is standard

Type:
Fresh or spent cell media from mammalian or microbial cultures and fermentations

Amino Acids	Alanine Aspartic acid Glycine Leucine Phenylalanine Threonine Valine	Arginine Glutamic acid Histidine Lysine Proline Tryptophan	Asparagine Glutamine Isoleucine Methionine Serine Tyrosine
Biogenic Amines	beta-Alanine L-Citrulline	GABA Methyl-L-histidine	Hydroxyproline Sarcosine
Vitamins	Choline Pyridoxine	Nicotineamide Thiamine	Pyridoxal
Dipeptides	Alanine-glutamine	Cystine	

THE DEVICE

Calibration:
Automated calibration and QC with consumable kits

System Interfaces:
Ethernet, USB port (2)

YOUR DATA

Output:
Values reported in "mM" concentration over 2.5 orders of magnitude linear dynamic range

Report format:
CSV and PDF

OPERATING CONSIDERATIONS

Ambient temperature:
15 - 25°C

Ambient humidity:
20 - 80% RH (non-condensing)

Solvent waste collected in dedicated vessel with level sensing

Condensate (water) collected in dedicated vessel with level sensing

Power requirements:
100-240 VAC, 50-60 Hz

Physical dimensions:
Height (22 in, 56 cm), width (13 in, 33 cm), depth (27 in, 69 cm), weight (83 lbs, 38 kg)

COMPLIANCE

Safety/Compliance: UL/CSA/IEC 61010-1 3rd Edition; CE marked; EU & China RoHS; EU REACH

SOFTWARE

Designed for operation in cGLP/cGMP, with support for 21 CFR Part 11-compliance

908devices
Rebel@908devices.com | 908devices.com


9:41 PM
Login
15/22 samples have been processed.
4:24
The system is currently processing LA1 HS metabolites.
PAUSE
Batch Analysis
Eric is running a batch that will be complete at 10:48 PM
The system is operational.