

Cell Culture Media Analysis Platform

C2MAP System



LC/MS/MS Cell Culture Media Analysis Platform

Capable of Obtaining Temporal Change Profiles for Up to 95 Culture Supernatant Components

Material Production

- Optimization of Culture Process
- Evaluation of Scale-up

Cell Therapy

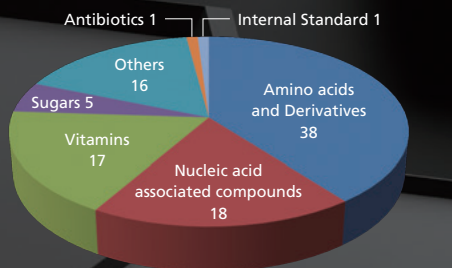
- Marker Search for Quality Evaluation

Culture Media

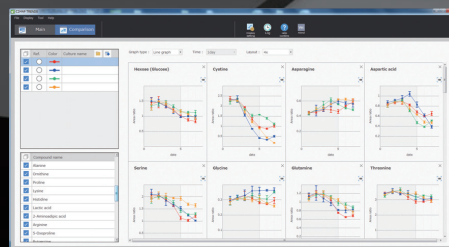
- Development of Culture Media
- Optimization of Media Composition



Automatic pretreatment of culture supernatant samples



Basal medium component / Secretory metabolite
Simultaneous LC/MS/MS analysis of up to 95 components



For component variation by C2MAP TRENDS™ Graph Display

Visualization of Temporal Change for Component Variations in Culture Media

The C2MAP™ System can be automated the process from pretreatment to measurement for culture supernatant samples and display temporal changes in the components as graphs.

With the optimized LC/MS/MS simultaneous analysis method, the system can be used for the optimization of culture conditions in animal cell cultures by monitoring the consumption and depletion of media components during culturing, as well as the variation in metabolites secreted from cells.

 Provides High-Quality Measurement Data with Ease




C2MAP-2030
Cell Culture Media Analysis Platform

Up to 65 samples can be pretreated and delivered to the HPLC automatically.

LCMS-8060/8050

Optimized cell culture profiling LC/MS/MS method package enables high-speed analysis of 95 components.

 Modules Required for Automated Pretreatment Can Be Operated Independently of LC/MS/MS



- The LC/MS/MS can be installed in a different room, allowing to be shared for other analyses.
- If culture samples cannot be taken out of the culture room, sample pretreatment can be performed in the culture room and measured by LC/MS/MS in another room.
- Separating the various components saves laboratory space.

Features of the C2MAP System

1

Automated Process from Pretreatment to Measurement for the Culture Supernatant Analysis

- The automated process allows analysis to be performed at night and on non-working days.
- The measurement workflow can be selected to match the actual culture.
- Seamless analysis and management from pretreatment to LC/MS/MS measurement can be achieved.
- Pretreated samples are stocked on a microplate automatically to enable re-measurement with ease.

2

Supports a Wide Range of Measurement Compounds and Culture Supernatant Samples

- A total of 95 components, including major basal culture media components for animal cells and secreted metabolites, can be simultaneously analyzed at high speed.
- Applicable to a wide range of cell culture media (iPS cells, ES cells, mesenchymal stem cells, T cells, and CHO cells)

3

Visualization of Component Variations in Culture Media

- Temporal changes in the components can be displayed as trend graphs.
- The results under multiple experimental conditions can be overlaid in the display, enabling comparative analysis.

4

Supports Flexible System Configurations

- By installing the automatic pretreatment unit separately, pretreatment and LC/MS/MS measurements can be performed in different rooms.



CHO cells, iPS cells
ES cells, T cells,
mesenchymal stem cells

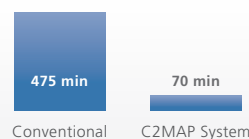
Culture Solution

Remove floating cells/dead cells by filtering or centrifugation (manual pretreatment is required beforehand).

Example of Operator Labor Time Comparison

*Labor Time for 65 Samples

Total



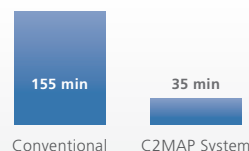
*An additional post-run analysis process is necessary. The post-run analysis is not included in the labor time calculation because the process is common to both the conventional method and the C2MAP system.

Performed by the C2MAP-2030
Cell Culture Media Analysis Platform

- Addition of internal standard
- Denaturation of proteins by adding organic solvents
- Stirring
- Suction filtration
- Sample delivery to the HPLC autosampler



Pretreatment-related Process



*For the C2MAP system, the time required for preparation of C2MAP-2030 is shown because it performs pretreatment process automatically.

*Time to remove floating cells and dead cells are not included.

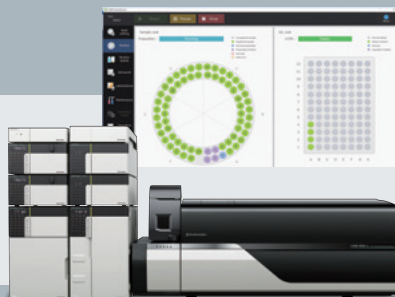
Performed by the SIL-30AC

- Automatic sample dilution
- Dispensing to the MTP



Performed by the LCMS-8060/8050
Prepared dedicated control software

- Measurement by LC/MS/MS
- Simultaneous analysis of up to 95 components using a cell culture profiling method



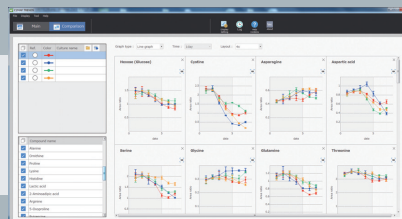
Sample Registration Process



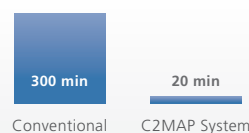
*LC/MS/MS analytical time is not included.

Performed by the C2MAP TRENDS

- Visualization of temporal changes in each component



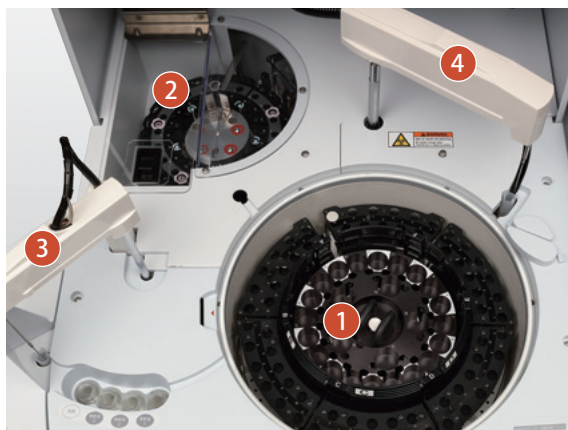
Graph Making Process



*Time comparison for graph making by C2MAP TRENDS and a spreadsheet is shown.

Start up the instrument / Place the samples and consumables

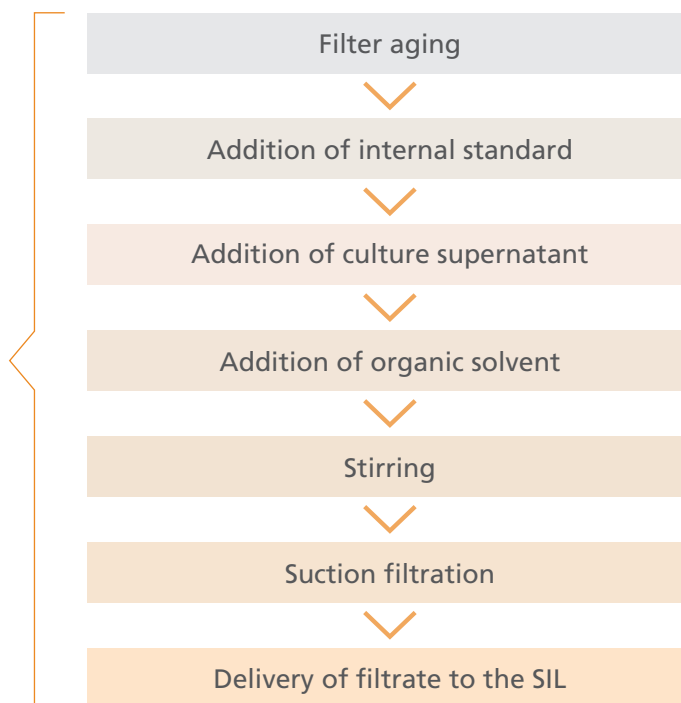
Up to 65 samples can be pretreated automatically.



- (1) Sample Rack and Reagent Table
- (2) Filter Rack
- (3) Sample Probe
- (4) Reagent Probe

The probe for the culture supernatant sample ((3) sample probe) and the probe for the internal standard and organic solvent used as the deproteinization agent ((4) reagent probe) are separated, limiting cross contamination between samples.

Process Automated with
the C2MAP-2030
Cell Culture Media Analysis Platform



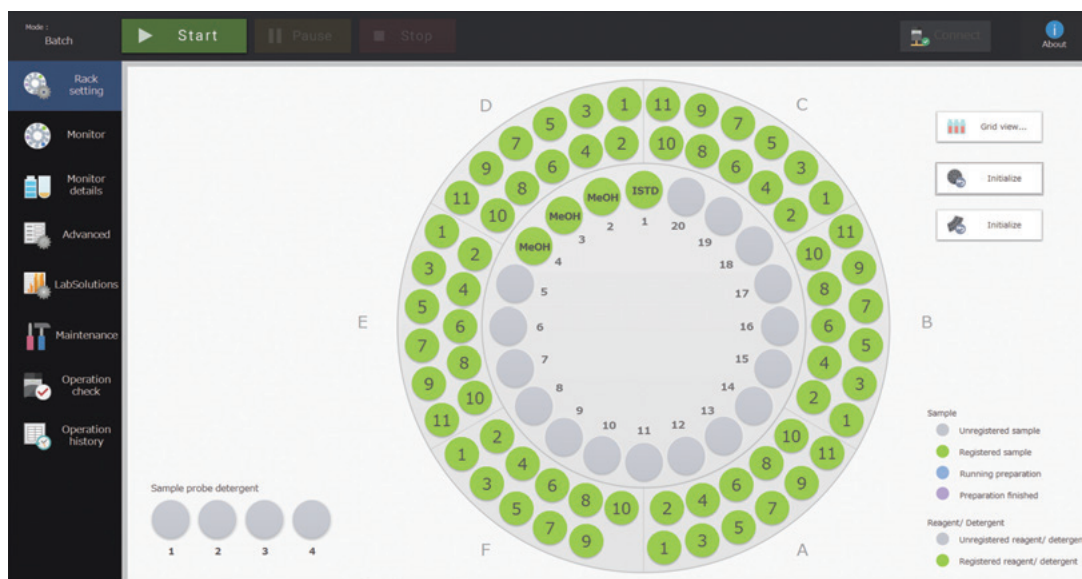
The sample is automatically delivered to the analytical instrument, enabling seamless analysis.

Register sample information in the control software

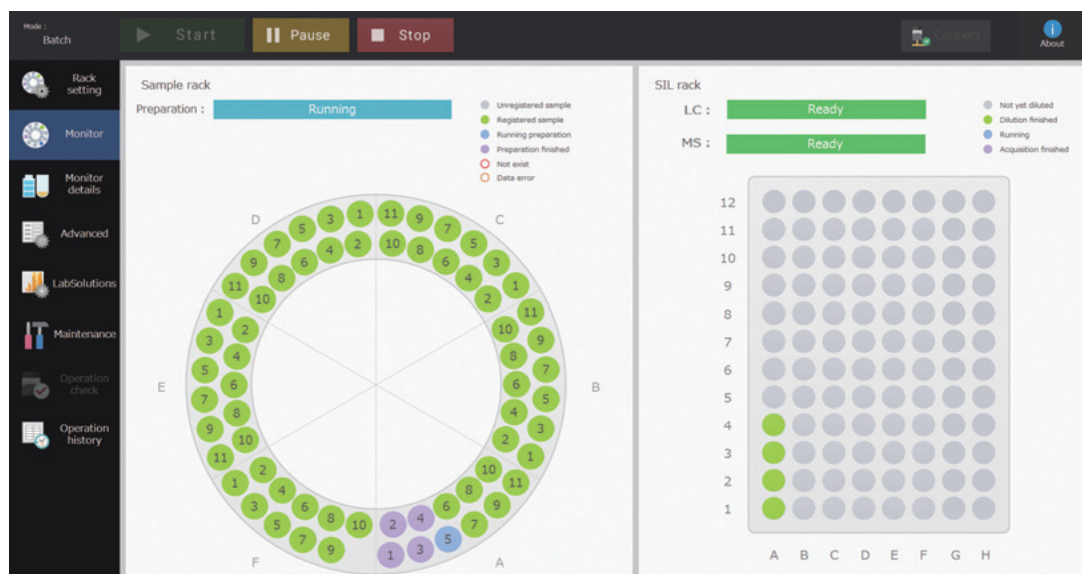
Start



The treated sample and the measurement results can be easily associated.



Everything from pretreatment to analysis can be carried out with the common sample ID.

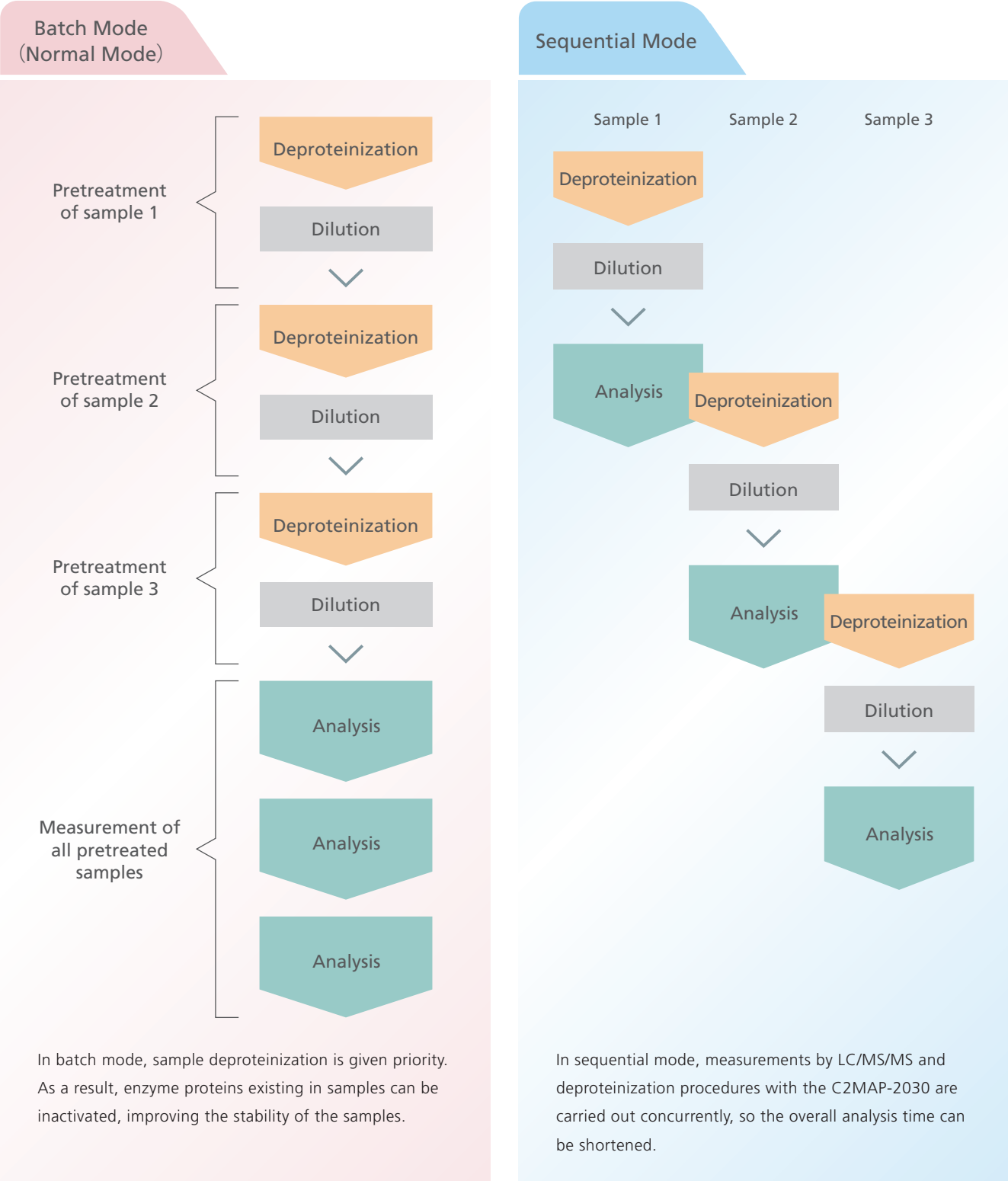


In the control software, the progress of pretreatment and analysis is easily confirmed.

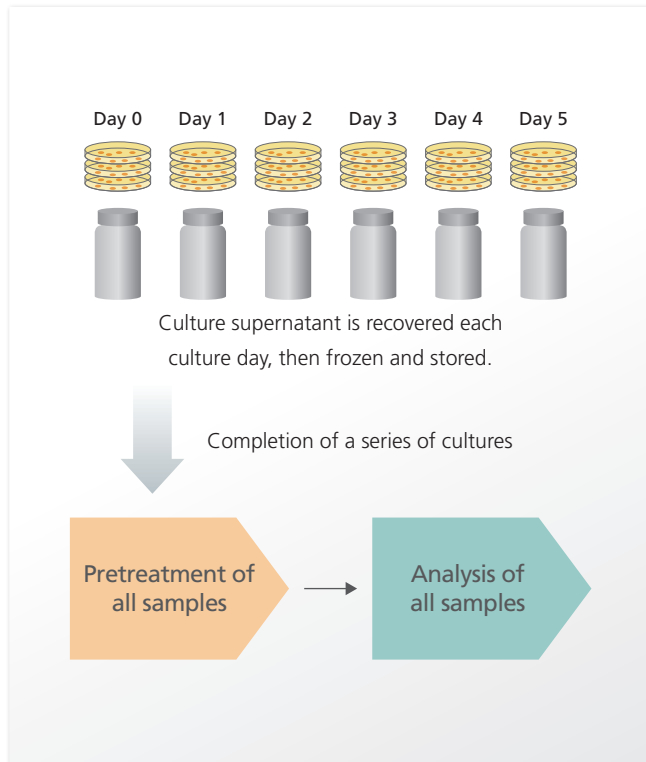
Using the dedicated software, analysis operations are performed intuitively. No complicated method settings are required.

The Optimal Workflow Can Be Selected

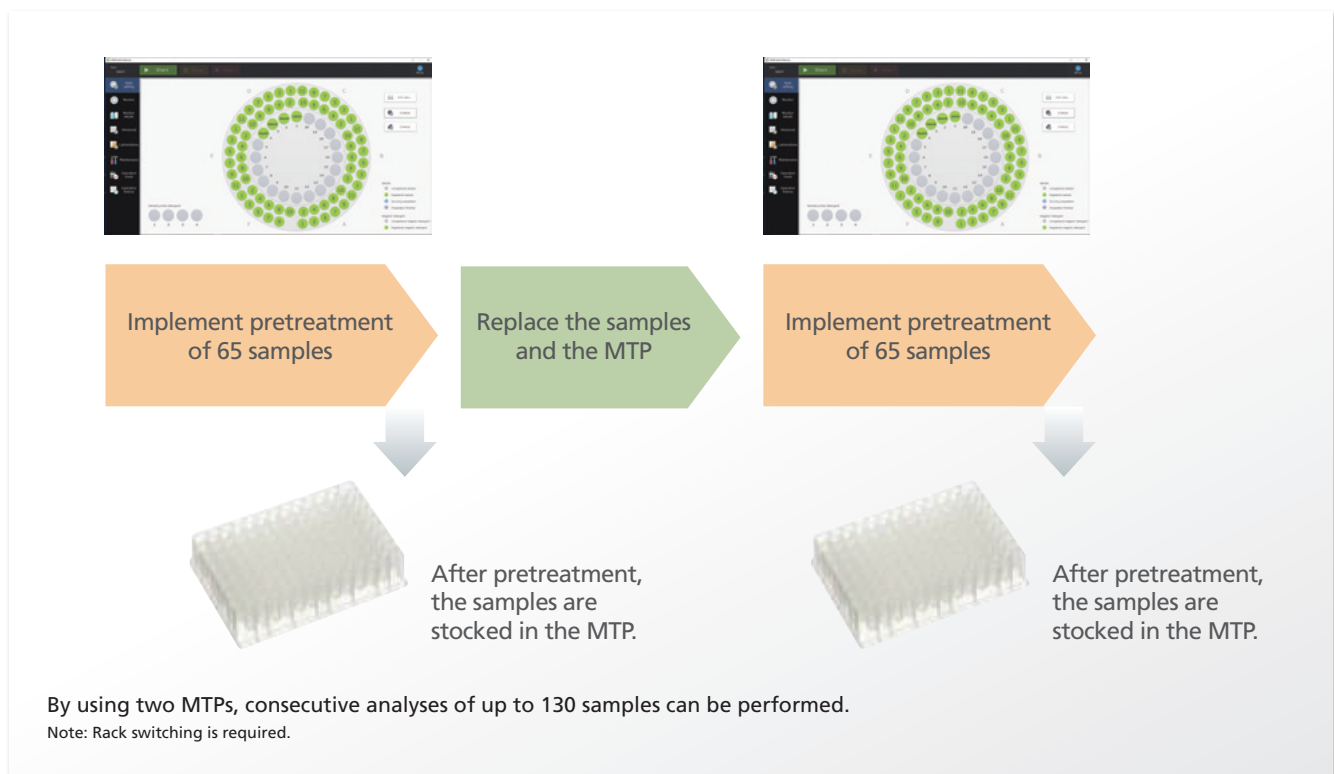
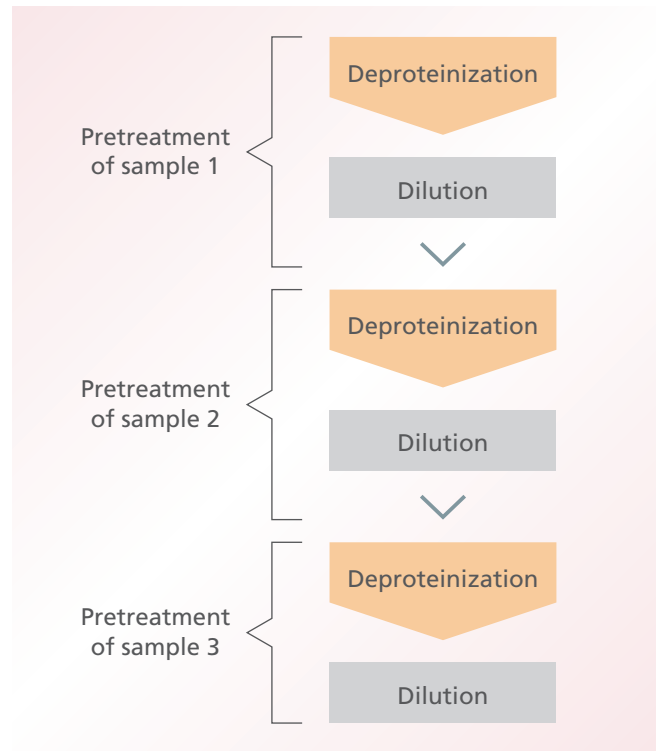
The C2MAP system supports two modes: **batch mode** (ordinarily used), which gives priority to the pretreatment of the culture supernatant samples placed, and **sequential mode** in which pretreatment and analysis are performed alternately for each sample.



Performing only the sample pretreatment process is possible in batch mode (normal mode).



Batch Mode (Pretreatment Only)



Supports a Wide Range of Measurement Compounds and Culture Supernatant Samples

The analysis method stored in the control software allows the simultaneous analysis of the following 95 components plus 2-Isopropylmalic acid.

Internal Standard	Amino Acid and Derivatives	Vitamins
2-Isopropylmalic acid	2-Aminoadipic acid	4-Aminobenzoic acid
	4-Aminobutyric acid	Ascorbic acid
	4-Hydroxyproline	Ascorbic acid 2-phosphate
Sugars	5-Glutamylcysteine	Biotin
Gluconic acid	5-Oxoproline	Choline
Glucosamine	Alanine	Cyanocobalamin
Hexose (Glucose)	Alanyl-glutamine	Ergocalciferol
Sucrose	Arginine	Folic acid
Threonic acid	Asparagine	Folinic acid
	Aspartic acid	Lipoic acid
	Citrulline	Niacinamide
Nucleic Acid Associated Compounds	Cystathionine	Nicotinic acid
Adenine	Cysteine	Pantothenic acid
Adenosine	Cystine	Pyridoxal
Adenosine monophosphate	Glutamic acid	Pyridoxine
Cytidine	Glutamine	Riboflavin
Cytidine monophosphate	Glutathione	Tocopherol acetate
Deoxycytidine	Glycine	
Guanine	Glycyl-glutamine	
Guanosine	Histidine	Others
Guanosine monophosphate	Isoleucine	2-Aminoethanol
Hypoxanthine	Kynurenine	2-Ketoisovaleric acid
Inosine	Leucine	3-Methyl-2-oxovaleric acid
Thymidine	Lysine	4-Hydroxyphenyllactic acid
Thymine	Methionine	Citric acid
Uracil	Methionine sulfoxide	Ethylenediamine
Uric acid	N-Acetylaspartic acid	Fumaric acid
Uridine	N-Acetylcysteine	Glyceric acid
Xanthine	Ornithine	Histamine
Xanthosine	Oxidized glutathione	Isocitric acid
	Phenylalanine	Lactic acid
Antibiotics	Pipecolic acid	Malic acid
Penicillin G	Proline	O-Phosphoethanolamine
	Serine	Putrescine
	Threonine	Pyruvic acid
	Tryptophan	Succinic acid
	Tyrosine	
	Valine	

It is confirmed that the C2MAP system can accommodate the following culture media and culture media additives.

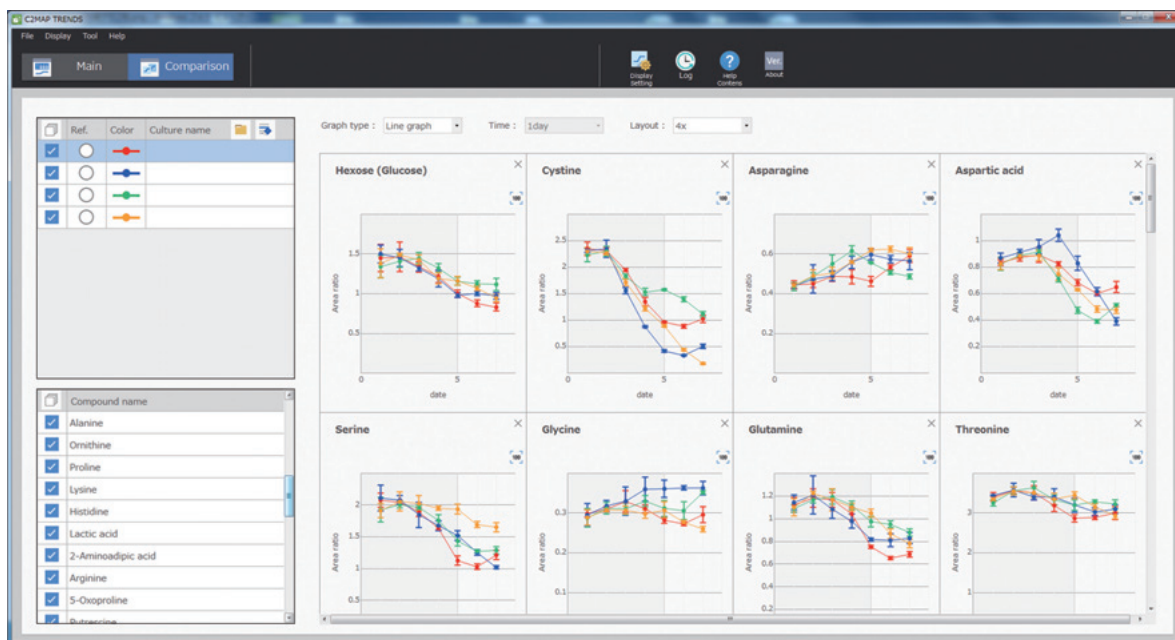
Cell Type	CHO cells	iPS/ES cells	T cells	Mesenchymal Cell Type
Culture Media	BalanCD® CHO	AK03N	X-VIVO™ 10	MSCBM™
	1×CD CHO	Essential-8™	X-VIVO™ 15	MesenPRO™
	EX-CELL® CHO	mTeSR™1/TeSR™-E8™	TexMACS™	Stempro®
Additives	Fetal Bovine Serum (100% v/v)			



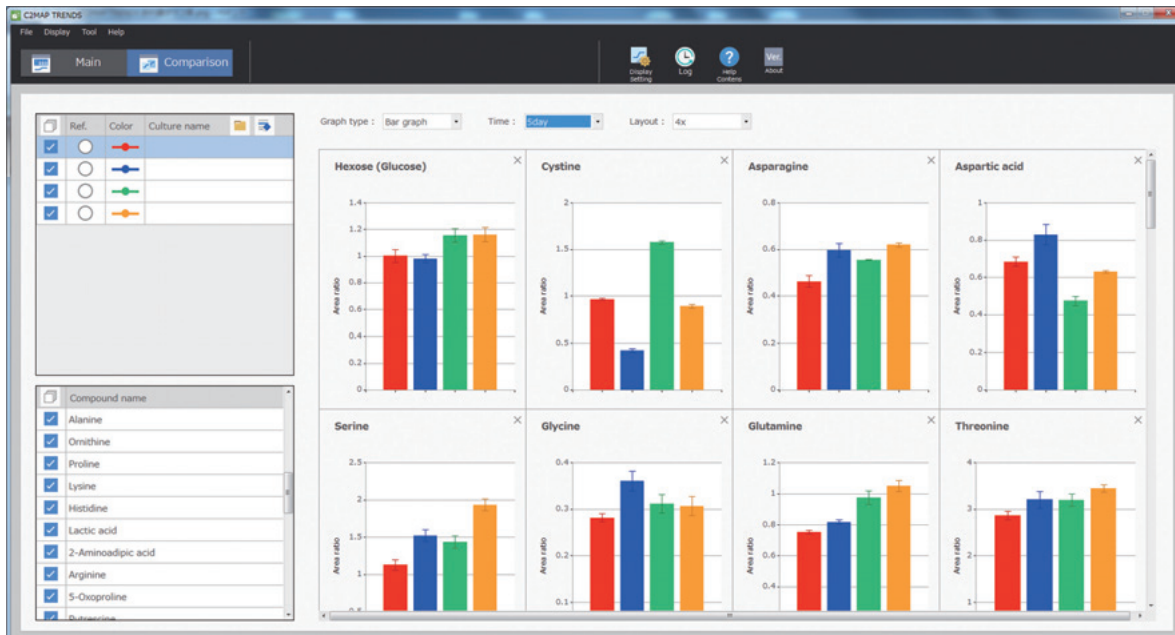
C2MAP TRENDS

For results obtained via LC/MS/MS, temporal changes in each component can be graphed by the dedicated viewer software. Analysts can monitor variations in metabolites secreted from cells and culture media components during cultivation, as well as display graphs of component comparisons with samples from different culture series. As a result, the consumption and depletion of culture media components, and changes in the amounts of metabolic components secreted from cells, can be observed, thereby providing useful insights about the optimal culture conditions and assessments of cellular status.

Temporal Changes in Measured Components



Measured-component Comparisons among Different Culture Series



System Flexibility Creates New Value

The automatic sample pretreatment unit and the LC/MS/MS unit can be installed in different laboratories for separate use. For example, everything up to sample pretreatment can be performed in the culture laboratory, while a shared LC/MS/MS can be installed in a separate laboratory.

The culture supernatant samples cannot be brought out of the culture laboratory, so sample pretreatment is implemented in the culture laboratory.

The LCMS can be shared for other analyses.

Culture laboratory



Pretreatment is implemented in the culture laboratory.

Analysis laboratory



LC/MS/MS measurement is performed in an analysis laboratory.

Note: Follow the regulations at the applicable company about bringing samples out of a culture laboratory.



Development of processes for antibody drugs



Cellular research on stem cells



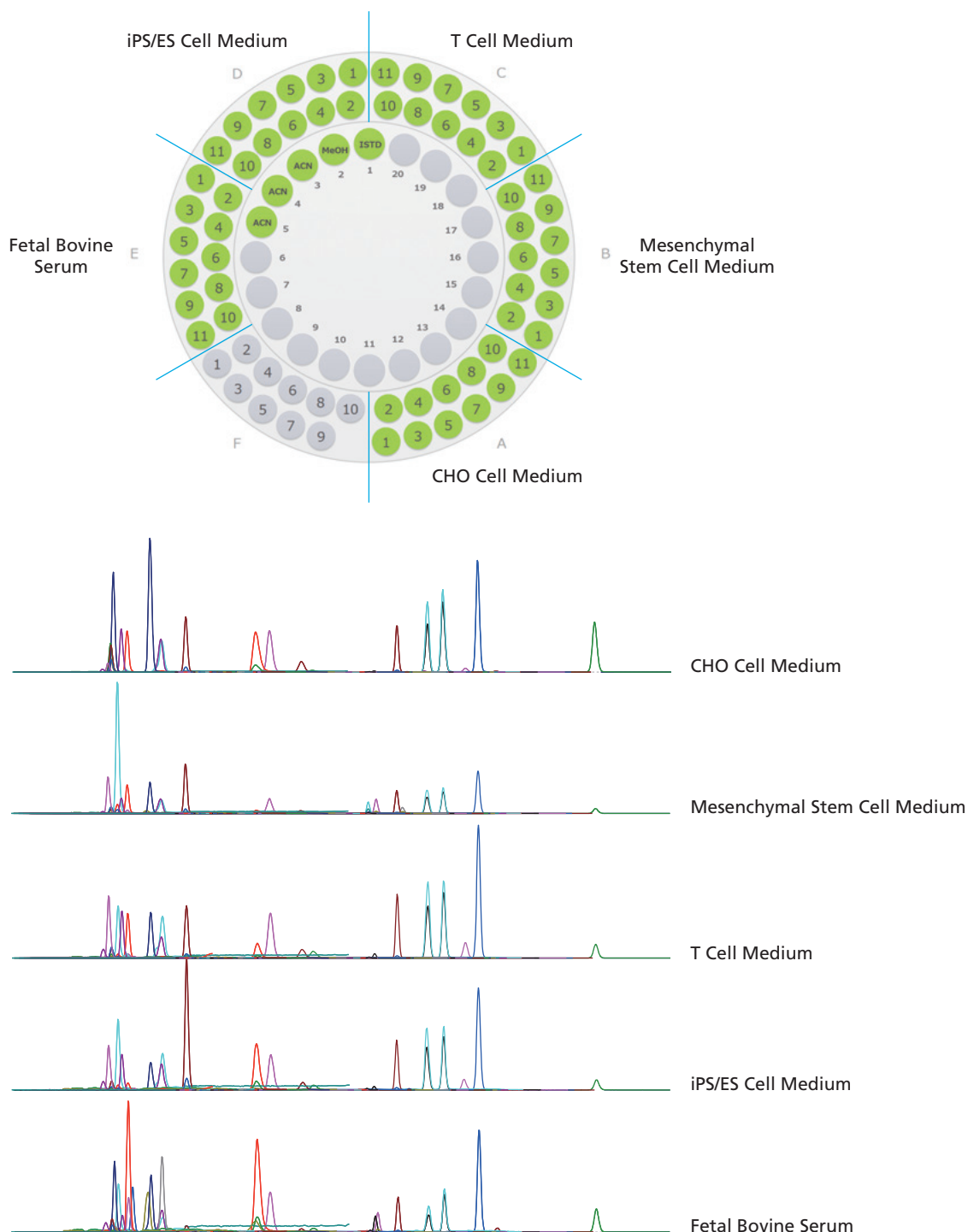
Cellular research on T cells



The LC/MS/MS can be shared for different projects.

Providing Recommended Pretreatment Conditions for Multiple Medium Types

By optimizing the pretreatment conditions, it is possible to pretreat a variety of cell types in a single pretreatment condition.

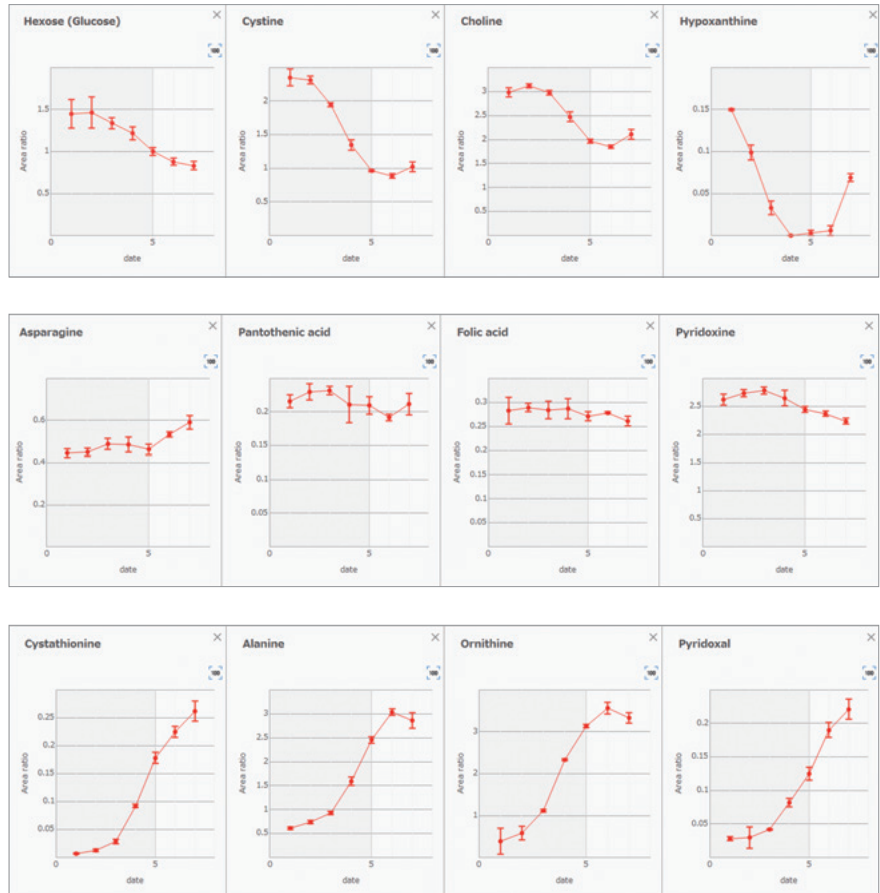


Pretreatment module can be shared and used for different projects.

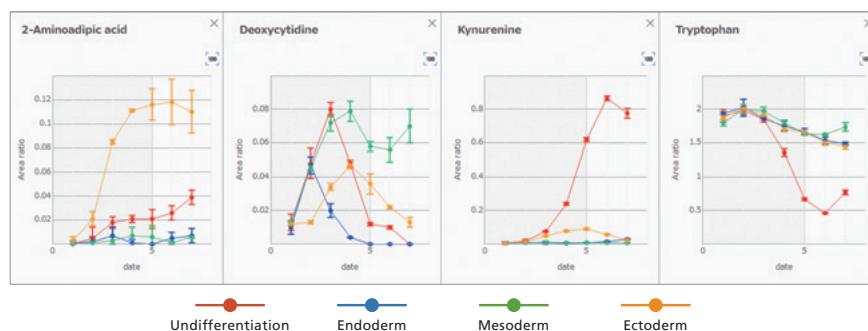
Application Data

Optimization of Culture Processes and Scaling Up of Culture Volumes

A culture supernatant after replacement of the culture media for undifferentiated human iPS cells was sampled. The temporal changes in the components in the culture supernatant were then monitored using the C2MAP system. The results suggested that hypoxanthine and some other components were depleted from Day 2 or later, despite replacing the culture media on a daily basis. In addition, the results indicated that asparagine, pantothenic acid, folic acid and pyridoxine maintained basically the same signal intensity throughout the culture period, suggesting that they are not easily consumed by the cells. Through multicomponent monitoring of the culture supernatant components, information can be obtained regarding which components are favored and consumed by cells, and which are depleted during the culture period. This information provides useful insights for optimizing the culture media composition and the culture process.



Next, the C2MAP system was used to compare the temporal changes in the culture supernatant components in undifferentiated human iPS cells and a model of cells deviated from the undifferentiated state (cytokines were added under undifferentiated culture conditions to induce differentiation of the various germ layers). As a result, it was possible to find compounds indicating the characteristic temporal changes in each model. Such compounds can become marker candidates for use in performing culture process management.



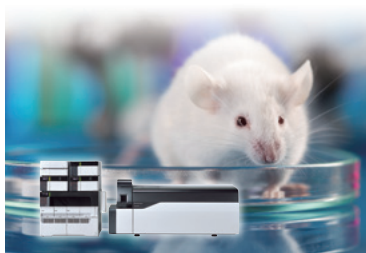
Cell Culture Profiling System

The combination of a High-Performance Triple Quadrupole Liquid Chromatograph Mass Spectrometer LCMS-8060/8050/8040 and the Cell Culture Profiling Method Package enables multi-component, simultaneous analysis of the components of the culture supernatant following manual pretreatment.



LC/MS/MS Method Package for Cell Culture Profiling LCMS/8060/8050/8040

A 95-component simultaneous analysis, including amino acids contained in culture media components and secreted metabolites, as well as sugars, vitamins, and organic acids, can be performed in 17 minutes per sample.



LC/MS/MS Method Package for Primary Metabolites Ver. 2

Either the ion pair method (55 components), which targets important compounds from the main metabolic pathways for biological samples, or the non-ion pair method (97 components), which targets the main amino acids and organic acids, can be selected to suit the instrument environment.



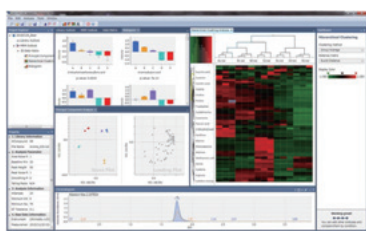
LC/MS/MS MRM Library for Phospholipids Profiling

Analysis targets the main phospholipids in biological samples. Phospholipid profiling is performed by combining two analysis methods: the phospholipid class determining method (422 components) and the fatty acid composition determining method (867 components).



LC/MS/MS Method Package for Lipid Mediators Ver. 2

This can perform simultaneous analysis of 158 components, including the main lipid mediators, such as eicosanoids, polyunsaturated fatty acid metabolites, and platelet activating factor.



Traverse MS™

This software is for the high-speed analysis of MRM data from multiple samples and multiple components. It can be used for principal component analysis and hierarchical clustering. This software is available from Reifycs Inc.

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