C297-E120

SHIMADZU
Excellence in Science

Cell Culture Media Analysis Platform

C2MAP System

New Cell Culture Media Analysis Platform for Fundamental Research and Process Development in Animal Cell Cultures

The C2MAP™ system measures component changes in a culture supernatant as culturing progresses using LC/MS/MS. This system can be used for a wide range of applications, from basic research of cell cultures including pluripotent stem cells (iPS cells and ES cells), mesenchymal stem cells, and antibody-producing cells, to scaling up of culture volumes and actual process development.

Obtain Temporal Change Profiles for up to 95 Culture Supernatant Components

C2MAP system can provide simultaneous analysis methods for up to 95 components, making it suitable for component analysis to determine cell growth and status quality. The dedicated C2MAP TRENDS viewer software can graph component variations across multiple conditions for easier comparative analysis.

Optimization of Culture Conditions

The system can provide useful insights 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.

C2MAP-2000

Cell Culture Media Analysis Platform

Deproteinization of culture media supernatant samples obtained from culture solution and the addition of internal standard substances are carried out automatically.

LCMS-8060/8050

Using the optimized cell culture profiling LC/MS/MS method package, 95 components are analyzed at high speed.

Nexera™ X2 (HPLC Unit)

Each sample is delivered automatically to the Nexera X2 autosampler, and analysis is started after automatic dilution.

PC Unit

The sequence of processes from pretreatment to analysis is performed by the C2MAP-2000 software in conjunction with LabSolutions LCMS. The results analyzed with LabSolutions LCMS can be displayed as trend graphs using C2MAP TRENDS software.

Features of the C2MAP System

1 Automated Process from Pretreatment to Measurement for the Culture Supernatant Analysis

- With an automated process from pretreatment to measurement, automatic analysis can 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 performed.
- 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.

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.)

Performed by the C2MAP-2000
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

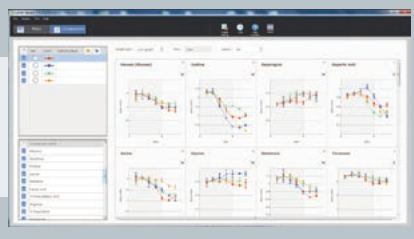
Performed by the SIL-30AC

- Automatic sample dilution
- Dispensing to the MTP

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

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

Performed by the C2MAP TRENDS

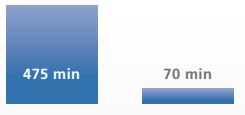


- Visualization of temporal changes in each component

Example of Operator Labor Time Comparison

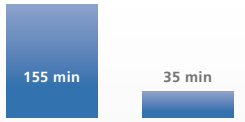
*Labor Time for 65 Samples

Total



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

Pretreatment-related Process

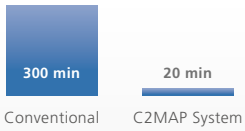


*In the case of C2MAP system, time required for preparation of C2MAP-2000 is shown because it performs pretreatment process automatically.
*Time to remove floating cells and dead cells are not included.

Sample Registration Process

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

Graph Making Process



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

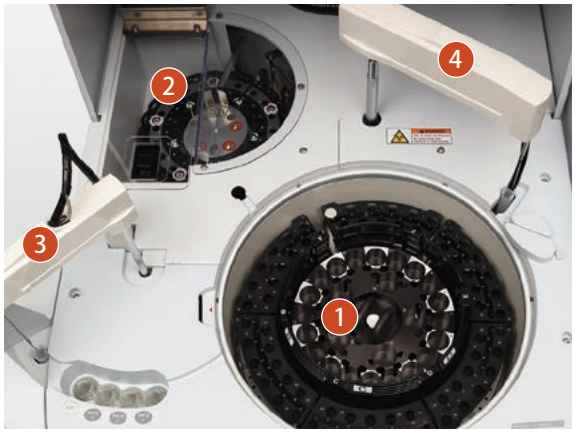
Automated Pretreatment Module for Cell Culture Media Analysis

C2MAP-2000

Cell Culture Media Analysis Platform

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-2000
Cell Culture Media Analysis Platform

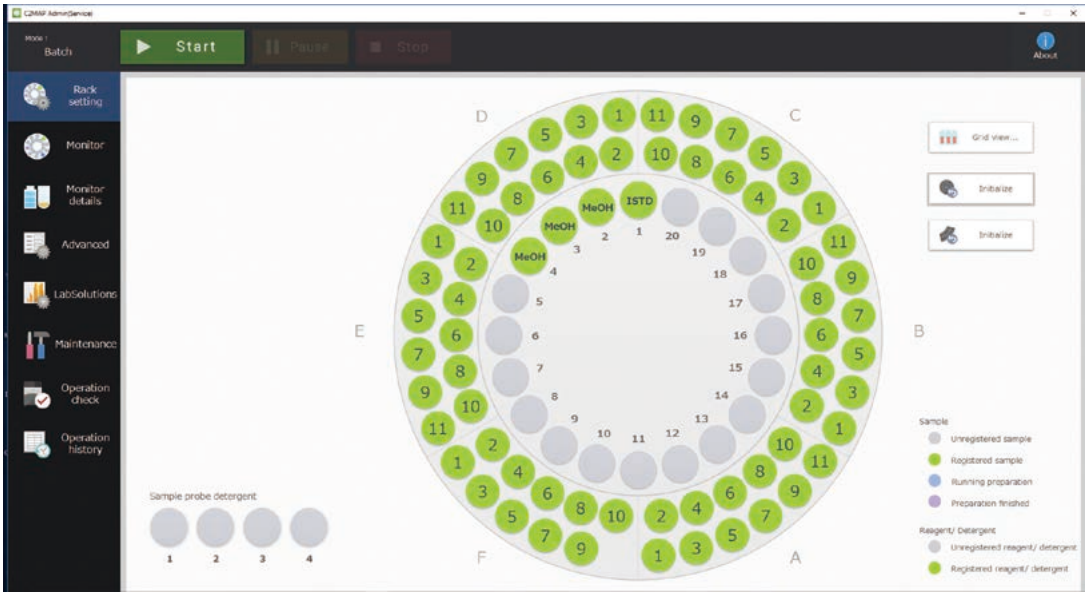
- Filter aging
- Addition of internal standard
- Addition of culture supernatant
- Addition of organic solvent
- Stirring
- Suction filtration
- Delivery of filtrate to the SIL

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

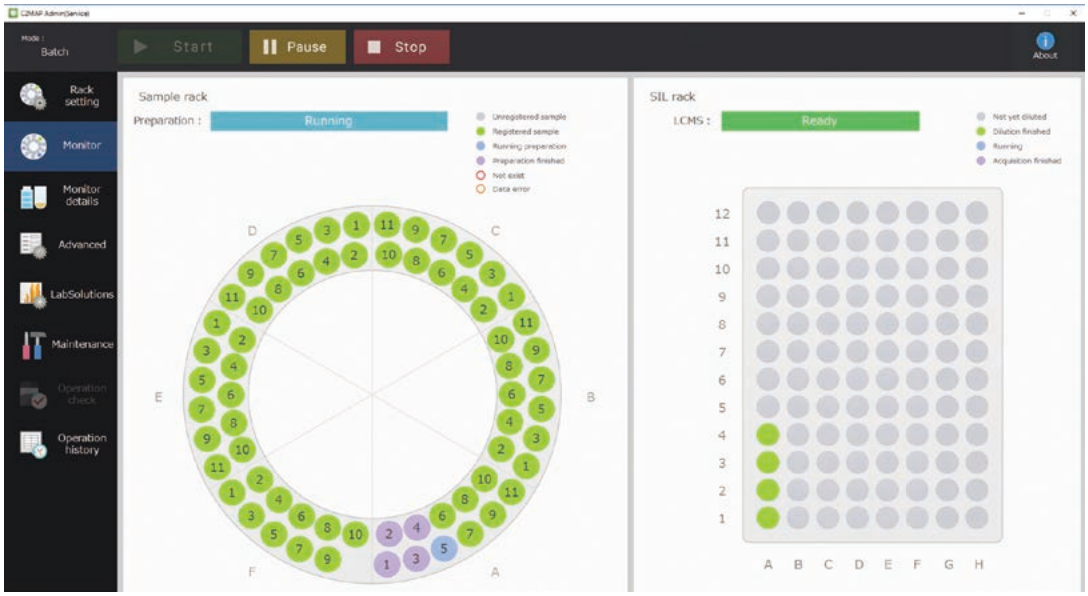
Register sample information in the control software.

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.



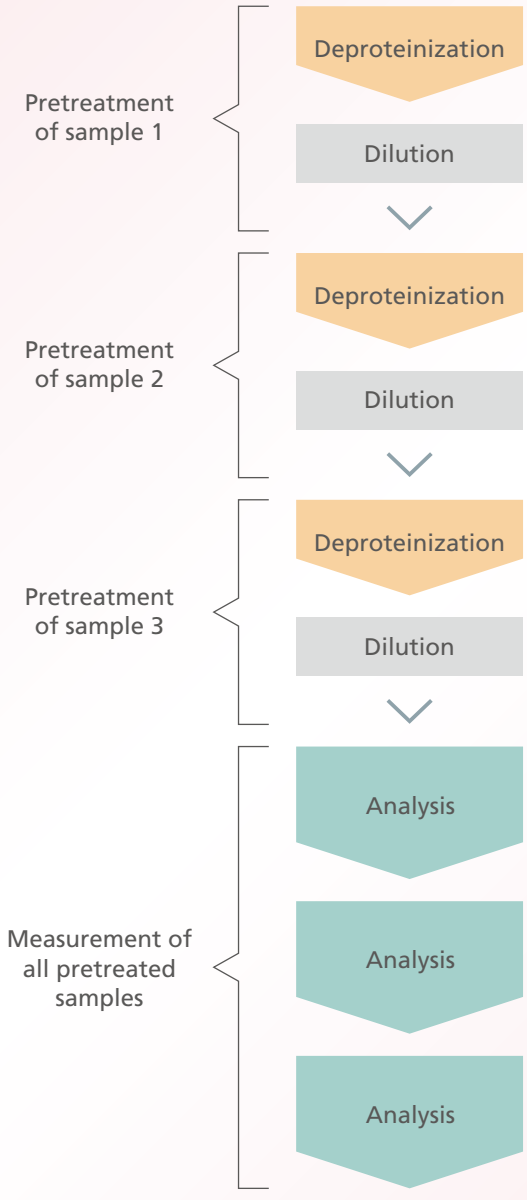
In the control software, the progress of pretreatment and analysis is easily confirmed.

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

The Optimal Workflow Can Be Selected

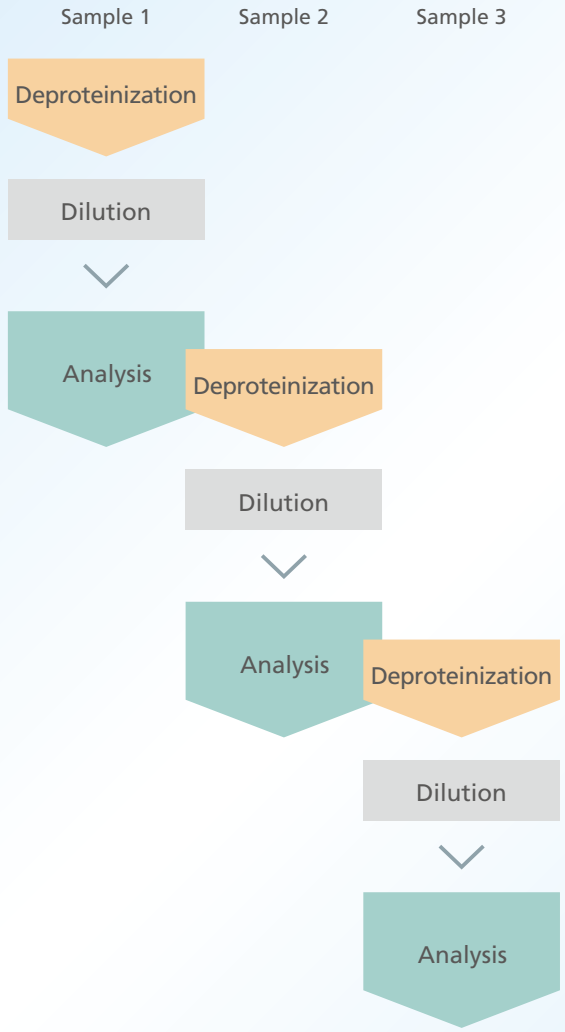
The C2MAP system supports two modes: Batch mode (ordinarily used) that gives priority to the pretreatment of the culture supernatant samples placed, and sequential mode in which pretreatment and analysis are performed alternately for each sample.

Normal Mode (Batch Mode)



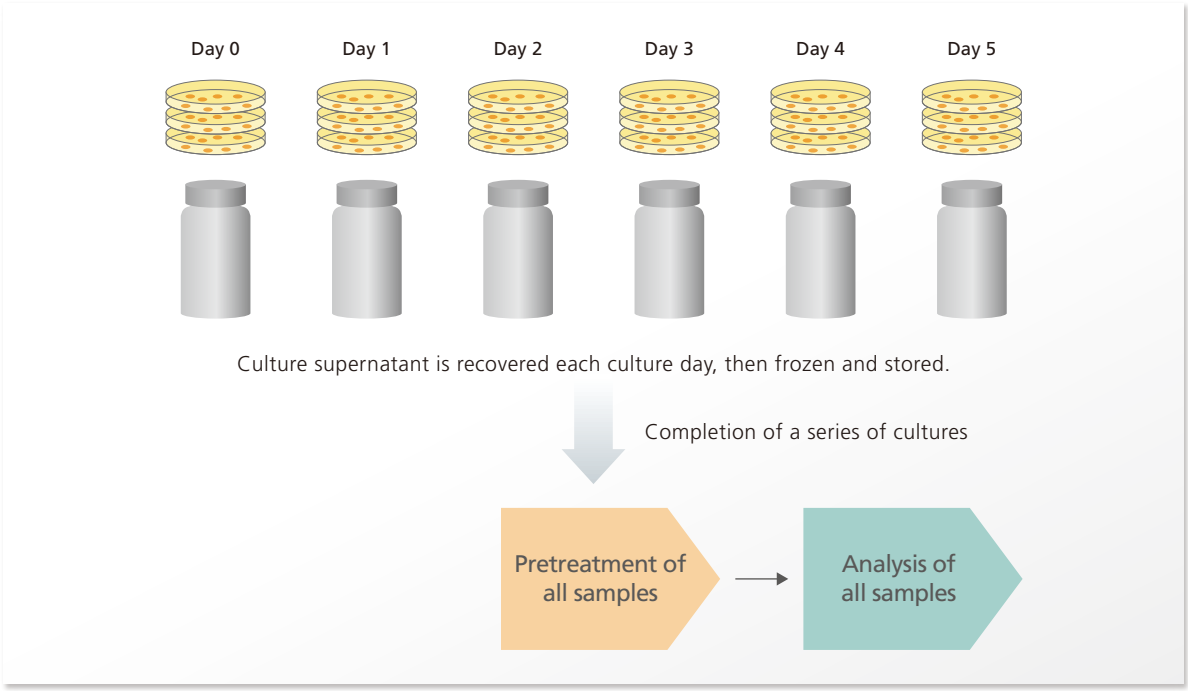
In batch mode, sample deproteinization is given priority. As a result, enzyme proteins existing in samples can be inactivated, improving the stability of the samples.

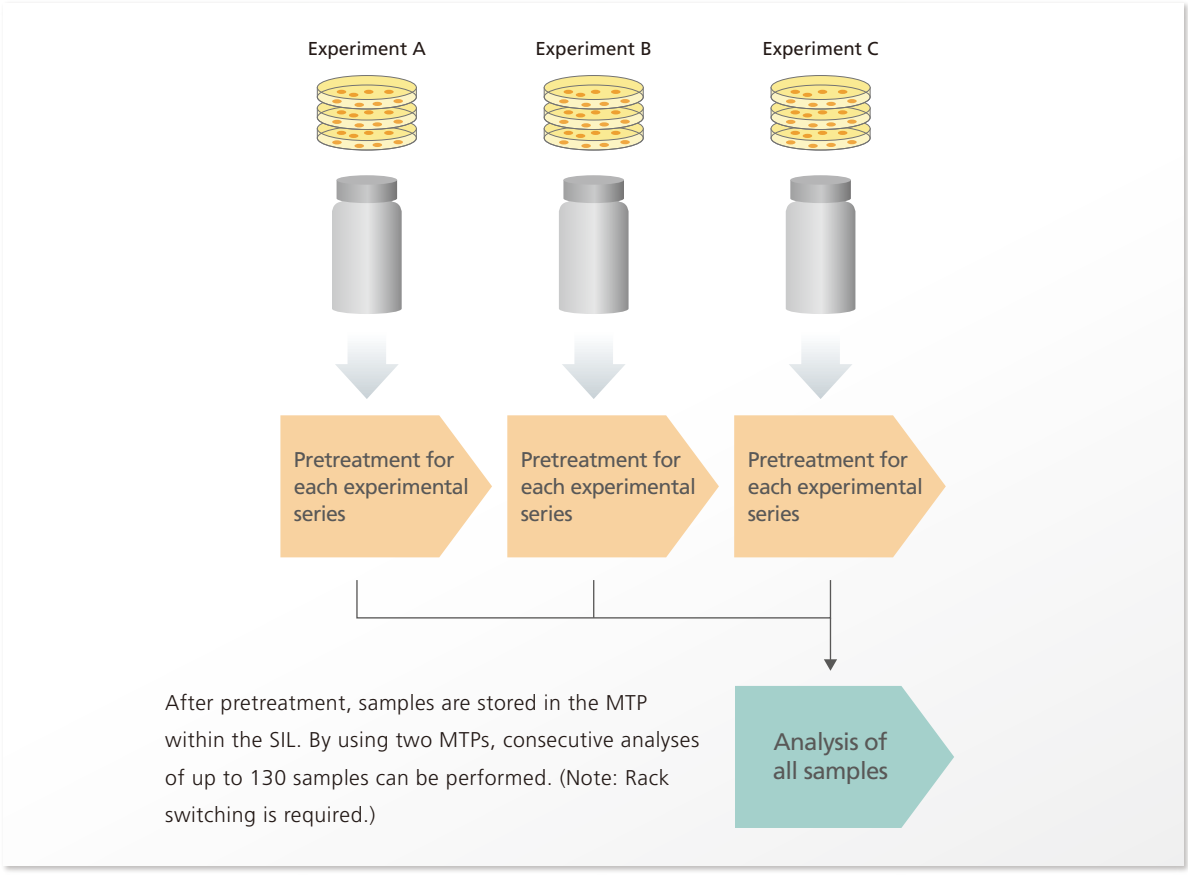
Sequential Mode



In sequential mode, measurements by LC/MS/MS and deproteinization procedures with the C2MAP-2000 are carried out concurrently, so the overall analysis time can be shortened.

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





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
2-Isopropylmalic acid

Sugars
Gluconic acid
Glucosamine
Hexose (Glucose)
Sucrose
Threonic acid

Nucleic acid associated compounds
Adenine
Adenosine
Adenosine monophosphate
Cytidine
Cytidine monophosphate
Deoxycytidine
Guanine
Guanosine
Guanosine monophosphate
Hypoxanthine
Inosine
Thymidine
Thymine
Uracil
Uric acid
Uridine
Xanthine
Xanthosine

Antibiotics
Penicillin G

Amino Acid and Derivatives
2-Aminoadipic acid
4-Aminobutyric acid
4-Hydroxyproline
5-Glutamylcysteine
5-Oxoproline
Alanine
Alanyl-glutamine
Arginine
Asparagine
Aspartic acid
Citrulline
Cystathionine
Cysteine
Cystine
Glutamic acid
Glutamine
Glutathione
Glycine
Glycyl-glutamine
Histidine
Isoleucine
Kynurenine
Leucine
Lysine
Methionine
Methionine sulfoxide
N-Acetylaspartic acid
N-Acetylcysteine
Ornithine
Oxidized glutathione
Phenylalanine
Pipecolic acid
Proline
Serine
Threonine
Tryptophan
Tyrosine
Valine

Vitamins
4-Aminobenzoic acid
Ascorbic acid
Ascorbic acid 2-phosphate
Biotin
Choline
Cyanocobalamin
Ergocalciferol
Folic acid
Folinic acid
Lipoic acid
Niacinamide
Nicotinic acid
Pantothenic acid
Pyridoxal
Pyridoxine
Riboflavin
Tocopherol acetate

Others
2-Aminoethanol
2-Ketoisovaleric acid
3-Methyl-2-oxovaleric acid
4-Hydroxyphenyllactic acid
Citric acid
Ethylenediamine
Fumaric acid
Glyceric acid
Histamine
Isocitric acid
Lactic acid
Malic acid
O-Phosphoethanolamine
Putrescine
Pyruvic acid
Succinic acid

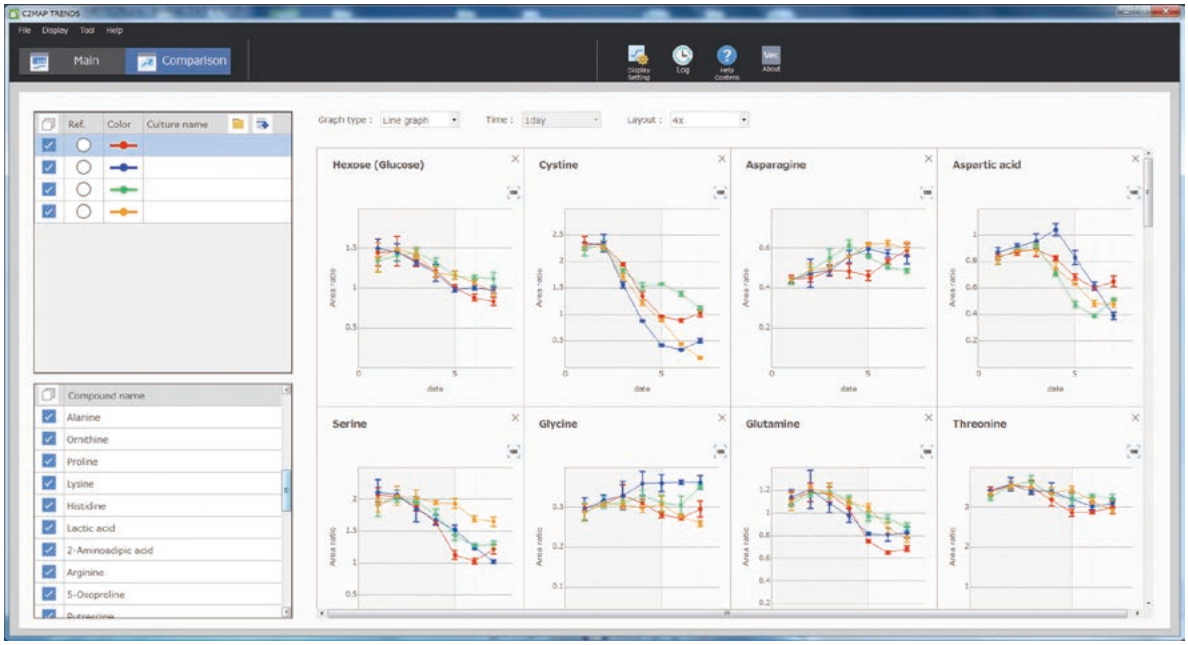
It is confirmed that the C2MAP system can accommodate the following culture media and culture media additives.

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)			

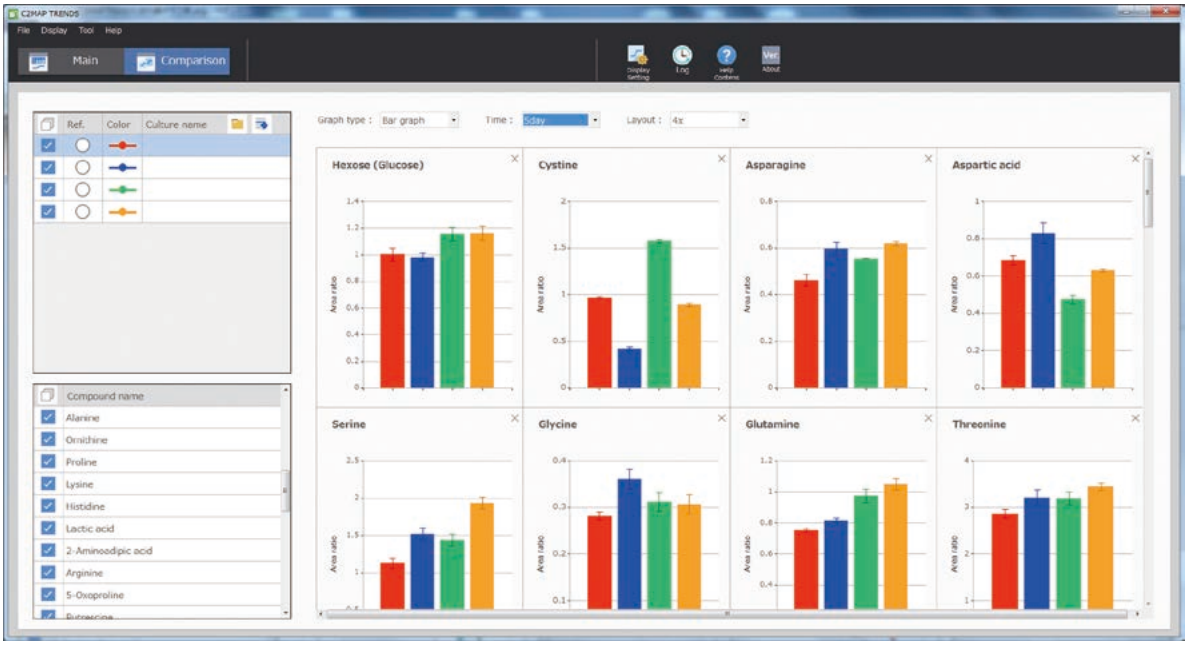
C2MAP TRENDS

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 into considerations of the optimal culture conditions and assessments of cellular status.

Temporal Changes in Measured Components



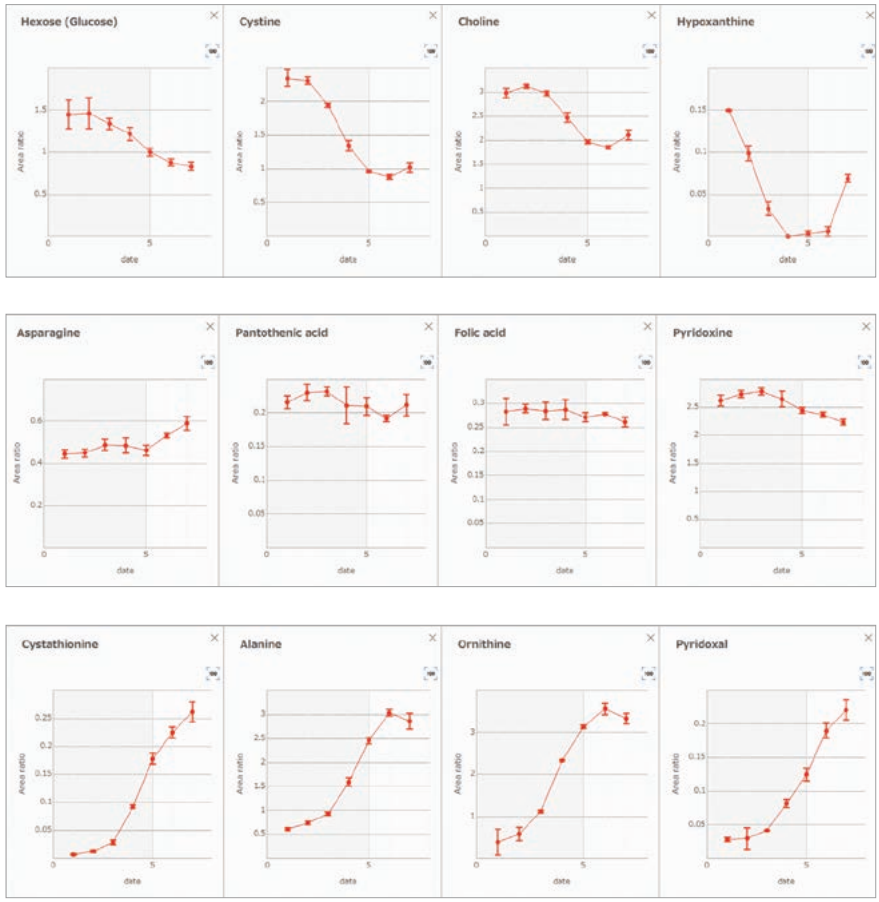
Measured-component Comparisons among Different Culture Series



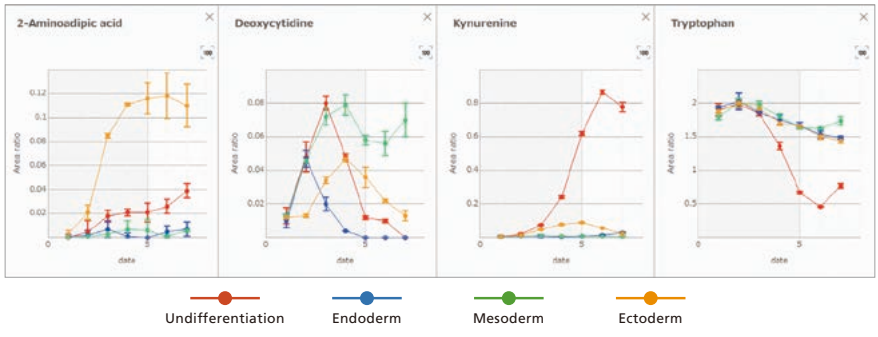
Application Data

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 into optimization of 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.



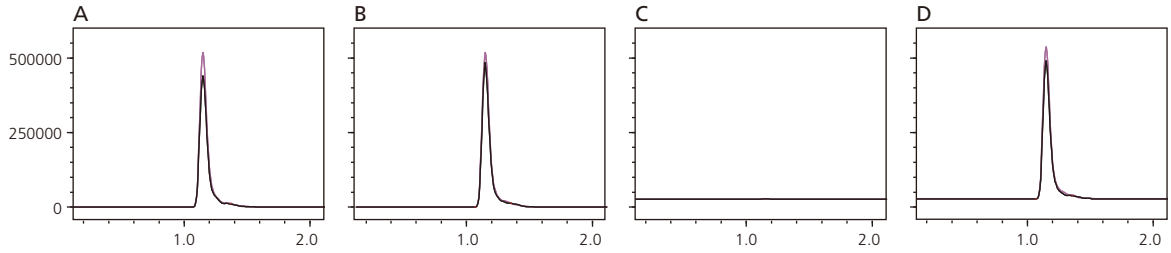
Qualitative Analysis of Basal Culture Media / Culture Media Additives

It is known that a difference in culture media additives has an impact on the culture results. In the example below, various types of Dulbecco's modified Eagle medium (DMEM) were analyzed by LC-MS/MS. The following table shows the differences in the DMEM culture media compositions noted in the product catalog.

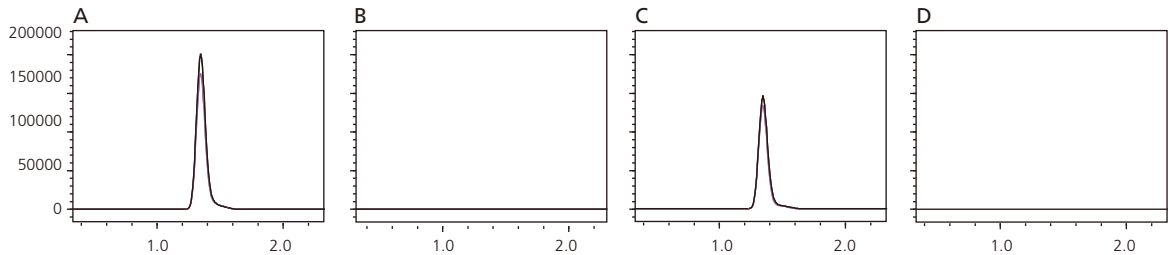
	A	B	C	D
Glucose	+	+	−	+
Glutamine	+	−	+	−
GlutaMAX	−	−	−	+

The results of the analysis match the content noted in the product catalog. When culture media and other raw materials are received, confirming the existence of the components that they should contain is important for stable substance production. Furthermore, creating calibration curves using standard products makes it possible to also quantify the concentrations, so the C2MAP system can be applied to the quantitative analysis of culture media.

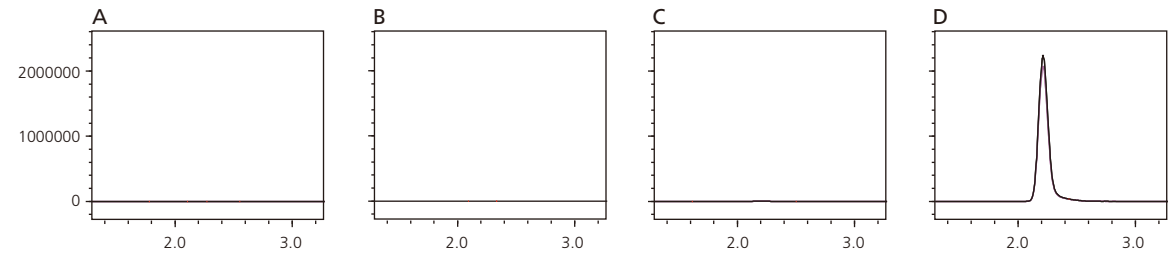
Glucose



Glutamine



GlutaMAX



Liquid Chromatograph Mass Spectrometer

LCMS-8060

Highest Sensitivity

A newly developed UF-Qarray boosts ion intensity but suppresses noise. By improving the ion sampling device, the ion guide, and vacuum efficiency, Shimadzu has achieved an unprecedented level of sensitivity in LCMS.

Fastest Speed

Shimadzu's proprietary technologies allow acquisition of up to 555 MRM channels per second, ultra-fast polarity switching, and ultra-fast scanning, all with high data quality.
UF scanning: Max. 30,000 u/sec
UF switching: 5 msec

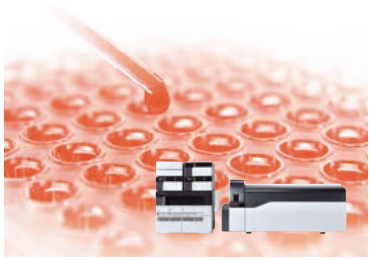
Fusion of Sensitivity and Speed

Liquid Chromatograph Mass Spectrometer

LCMS-8050

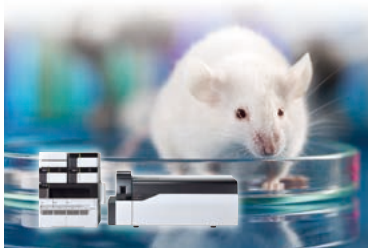
The LCMS-8050 incorporates more evolved UF Technology, so measurements can now be performed even faster, without sacrificing data quality. The system can be used in a wide range of fields for a variety of applications, such as quantitative analysis which requires high sensitivity, multicomponent simultaneous analysis, and screening.

LC/MS/MS Option Software



LC/MS/MS Method Package for Cell Culture Profiling

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

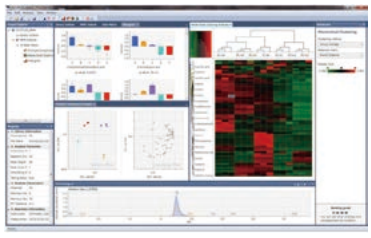
Either the ion pair method (55 components), which targets important compounds from the main metabolic pathways for biological samples, or the non-ion pair method (97 components), which targets the main amino acids and organic acids, can be selected to suit the instrument environment.

LC/MS/MS 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.

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).



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.

Shimadzu Corporation
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