

# Application News

## No. B98

### MALDI-TOF Mass Spectrometry

## Simple Evaluation of Cultured Cells: Evaluation of Differentiation State of iPS Cells Using Benchtop MALDI-TOF MS

The growth condition and differentiation state (differentiated or undifferentiated) of cultured cells are important evaluation items in regenerative medicine and basic research. If these cell evaluations can be conducted in a simple manner by mass spectrometry, evaluation time and complicated work can be significantly reduced.

Therefore, in order to examine the possibility of determining the state of cells by mass spectrometry, we conducted an automatic analysis of iPS cells and their culture media by AuraSolution™ using the Shimadzu MALDI-8020 benchtop linear MALDI-TOF mass spectrometer, and attempted a preliminary analysis by the statistical analysis software eMSTAT Solution™. The results are reported in this article.

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### ■ Preparation of Analysis Samples

As the cultured cell samples and culture medium samples, the iPS cells and culture media at various stages of culturing shown in Table 1 were used. During culturing, one-half of the culture medium was replaced with fresh medium every other day. Using quantitative PCR (polymerase chain reaction), we compared the relative expression amounts of the differentiated/undifferentiated marker OCT3/4 and NANOG (undifferentiated marker), SOX1 (exoderm marker), and SOX17 (endoderm marker) with the cells at various stages of culturing. The results suggested that differentiation progresses from the undifferentiated state through a transition state from

undifferentiated spheroids to differentiated spheroids and then adherent differentiated cells in that order.

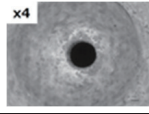
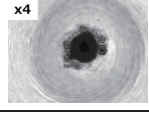
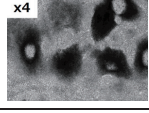
In the cell measurements by mass spectrometry, after recovering and washing the respective cultured cells twice with a PBS buffer, pellets were obtained by short-time ultrasonic cleaning under 10 % trichloroacetic acid and centrifuging at 10,000 xg for 1 min. The recovered pellets were suspended in a small amount of an acetonitrile solution, and the suspension (1 μL) was mixed with CHCA (α-cyano-4-hydroxycinnamic acid, 1 μL), which had been dissolved to a concentration of 5 mg/mL in the matrix solution (50 % acetonitrile solution containing 0.1 % trifluoroacetic acid). This mixture was placed on the MALDI target plate, dried, and then measured.

In the culture medium measurements, the full amount of a mixture of the culture supernatant (0.5 μL) and the CHCA solution (same as above, 0.5 μL) was placed on the MALDI target plate, dried, and measured.

### ■ MALDI-TOF MS Measurement of Analysis Samples

The analysis samples placed on the MALDI target plates were inserted into a MALDI-8020 benchtop linear MALDI-TOF MS, and mass spectra were obtained by automatic analysis using the automatic analysis software Sample Station™/AuraSolution. Table 2 shows the measurement parameters in the automatic analysis. Fig. 1 shows an example of a mass spectrum.

**Table 1 List of Analysis Samples**

Sample	Culture medium	Culture time (days)	Morphology	n number
Undifferentiated spheroid	Essential 8™	7 days		6
Differentiated spheroid	Essential 8™ + FBS (fetal bovine serum)	7 days		6
Adherent differentiated cell	Essential 8™ + FBS + gelatin coat	7 days		6
Undifferentiated culture medium (D2)	Essential 8™	2 Days	-	6
Undifferentiated culture medium (D7)	Essential 8™	7 days	-	6
Differentiated culture medium (D2)	Essential 8™ + FBS	2 Days	-	6
Differentiated culture medium (D7)	Essential 8™ + FBS	7 days	-	6
Differentiated culture medium gelatin coat (D2)	Essential 8™ + FBS + gelatin coat	2 Days	-	6
Differentiated culture medium gelatin coat (D7)	Essential 8™ + FBS + gelatin coat	7 days	-	6

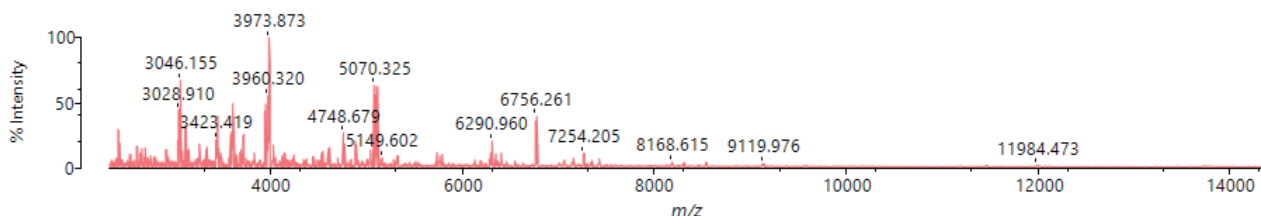
**Table 2 MALDI Data Acquisition Parameters**

Cell Measurement	
Tuning	linear
Polarity	positive
Mass range	2,000-30,000 Da
Laser rep. rate	200 Hz
Accumulation rate (laser shots/profile)	20
Profiles	1156
Sampling method	Raster
Culture Supernatant Measurement	
Tuning	Linear
Polarity	Positive
Mass range	2,000-30,000 Da
Laser rep. rate	200 Hz
Accumulation rate (laser shots/profile)	20
Profiles	676
Sampling method	Raster

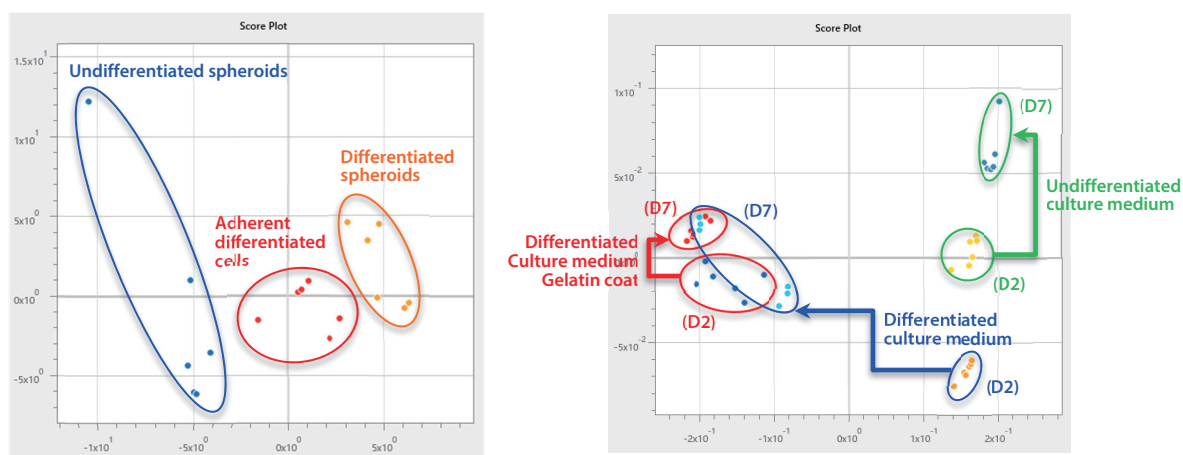
### ■ Analysis of MS Data by eMSTAT Solution

As the result of the analysis of the MS data obtained by automatic analysis by the eMSTAT Solution software, the undifferentiated spheroids, differentiated spheroids, and adherent differentiated cells cultured for seven days in the respective conditions of culturing could be grouped respectively as shown in Fig. 2. Regarding the culture media, in addition to grouping by culture time (days), it was found that the results of differentiated culture medium (D7) of the seventh day of culturing, which formed spheroids, formed a group that was close to the differentiated culture medium gelatin coat (D2) and differentiated culture medium gelatin coat (D7), which were the culture medium of the adherent differentiated cells.

It is thought that these results show the possibility of simpler and faster evaluations of the growth condition and differentiation state of cells by using a combination of an automatic analysis software suitable for multi-sample processing and the statistical analysis software eMSTAT Solution, based on the MALDI-8020 simple benchtop linear MALDI-TOF mass spectrometer.



**Fig. 1 Example of Mass Spectrum of iPS Cells Obtained by Direct Measurement**



**Fig. 2 Results of Multivariate Analysis of MS Data by eMSTAT Solution**  
Left: iPS Cells, Right: Culture Supernatant

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