TECAN

Total RNA Isolation System

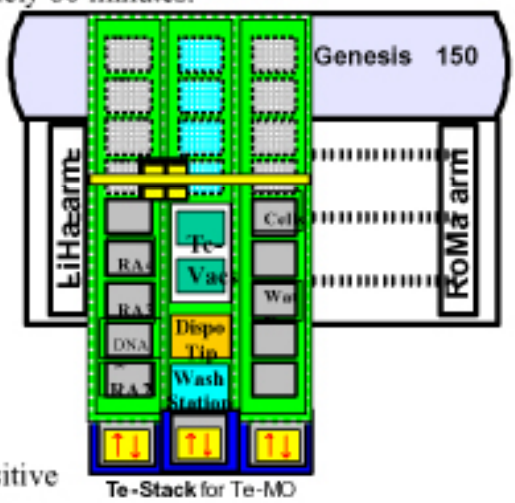
A fully automated procedure for the preparation of total RNA from cells using Macherey-Nagel's NucleoSpin 96 RNA kit on Tecan Genesis Workstation/ Te-MO.

Isolation of TotalRNA from the mouse fibroblast cell line L929 and the mouse melanoma cell line B16F10 were determined on Tecan GenesisWorkstation. Pipetting is performed by the Te-MO which has 96 channel pippeting head, and vacuum is performed by the Te-Vacs on Te-MO(customized). Disposable tip suppl, transportation of the filter plate, insertion of the collection plate into the Te-Vacs are fully automated. This system allows the processing of samples in a 96 well plate within approximately 60 minutes.

〈Methods〉

・Instrumentation

Genesis RWS150/8 with RoMa (RoboticManipulator), 8 channels with fixed Tip ;Te-MO (Multi-Channel Pipetting Option) ;Te-VacsB on Te-MO ; Software :TECAN GeminiV4.0



・Cells

L929 Mouse connective tissue, fibroblast like, TNF α sensitive

B16F10 Mouse GM-CSF producing B16 melanoma F10. Used for the model of gene treatment of cancer.

※Cells were dispensed in a 96 well MTP as following cell number per well and centrifuged (1500G×3min). (1×10^6Cells/well, 1×10^5Cells/well, 4×10^5Cells/well) After centrifugation, medium were removed and immediately Lysis Buffer(RA1; RNAse inhibitor)was added with a manual pipetter. These cells were tested using the Genesis Workstation within one day.

・Cells were generously supplied by associate professor Yutaka Miura, Tokyo University of Agriculture and Technology.

RNA concentration and purity were quantified by absorbance reading (OD260/280nm) using TECAN Genious.

〈Typical Results〉

Using 96 channel pippeting head Te-MO, and vaccum unit Te-Vacs on Te-MO, the samples in a 96 well plate were processed within approximately 60 minutes.The Yield and purity is shown below.(In this study,the kit is not used by the standard protocol .The result is the reference due to the delay of the extraction after adding the RNAse inhibitor caused by the transportation of the samples.)

	Number of cells used	Yield (μg)	$A_{260/280}$
L929	1×10^6	3.52	2.04
L929	1×10^5	1.94	1.91
B16F10	4×10^5	6.36	1.97

※Total RNA was extracted after 2.5hrs of the adding the Lysis Buffer.

〈Conclusion〉

In this study,samples which had time lag caused by the transportation,were determined for the RNA extraction.Comparing these result,it is recognized that the yield of total RNA decreased by the time lapse. This suggests that yield of total RNA will be increased if the cells were immediately supplied for the extraction after centrifugation. It is effective for the automated total RNA extraction that GWS is placed for the exclusive use for the total RNA extraction to get the high yeild. This can also effective on the inhibition of the RNAse contamination.Using 96 channel pippeting head Te-MO,the pipetting time is shortened. This is particularly effective for this kind of time influenced application on the yield like the total RNA ectraction.