



Application

## Sample Preparation for Quantitative Analysis of Integrin Expression

Product

FastGene® RNA Premium Kit (FG-81050, FG-81250)

Manufacturer

NIPPON Genetics EUROPE

The following data has been posted by the courtesy of Otani Haruki-sama, Frontier Laboratory for Integrated Treatment and Development, Graduate School of Biomedical & Health Sciences, Hiroshima University, Japan.

Overview

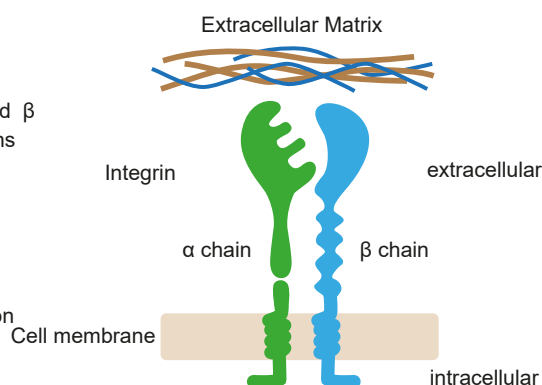
My target was to investigate the role of integrin in iPS cells and to confirm the type of integrin being expressed by qPCR. I decided to use SW 480, which was reported to be expressing these integrins as a control, so that we can get a detailed impression of several types of integrins that we are paying attention to.

At that time sample preparation and RNA extraction was carried out using the FastGene® RNA Premium kit.

Q: What is integrin?

A cell adhesion molecule on the surface of the cell. The structure consists of two subunits, an  $\alpha$  chain and a  $\beta$  chain. Each protein molecule consists of different  $\alpha$  and  $\beta$  chains, so that there are many combinations which leads to various types of integrins depending on the combination of  $\alpha$  and  $\beta$  chain.

Each integrin has a function as a receptor of a specific extracellular matrix and is based on cell adhesion with an extracellular matrix (or cell), and it is composed of tissues in cell spreading, migration, proliferation, development, transmission of information from extracellular, cancer metastasis, repair of tissues, blood coagulation and so on.



Experimental conditions

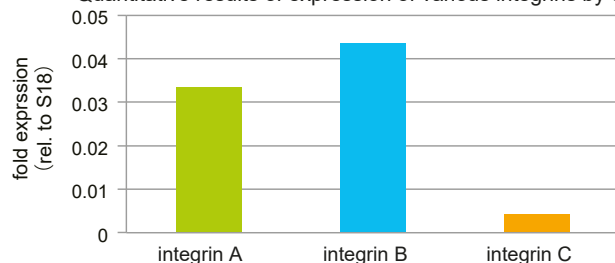
- Sample
  - Human colon carcinoma derived cell line (SW480)  $6 \times 10^6$  cells
  - ※ 10 cm dish was up to 90% confluent
- Pretreatment method: Wash with 1 x PBS (-)
- Elution buffer RE amount: 20  $\mu$ L
- Yield / purity measuring device: NanoDrop™ 2000 (Thermo Fisher Scientific)
- Reverse Transcription Reaction Conditions
  - Input RNA amount: 2.5  $\mu$ g (20  $\mu$ L per reaction)
  - Reverse transcriptase: ReverTra Ace (TOYOBO)
- Real-time PCR conditions
  - Input cDNA amount: 1  $\mu$ L of reverse transcription reaction solution (20  $\mu$ L for 1 reaction)
  - Real-time reagent: Brilliant III Ultra-Fast SYBR Green QPCR Master Mix (Agilent Technologies)
  - Real-time device: 7300 Real-Time PCR System (Applied Biosystems)

Result

RNA extraction result

Sample amount	Yield	Purity	
		$A_{260}/A_{280}$	$A_{260}/A_{230}$
$6 \times 10^6$ cells	3.8 mg/ml (20 $\mu$ L Elution)	2.12	2.16

Quantitative results of expression of various integrins by qPCR



Customer comment

Sometimes it is necessary to extract RNA from a large amount of sample, but with this kit the operation is completed in about 30 minutes and the price is also very competitive. The kit is useful because it reaches a good yield. I also felt the merit of your company's RNA extraction kit from various aspects even from the fact that RNA can be extracted without problems in purity and concentration.