



QuickGene Series Application Guide

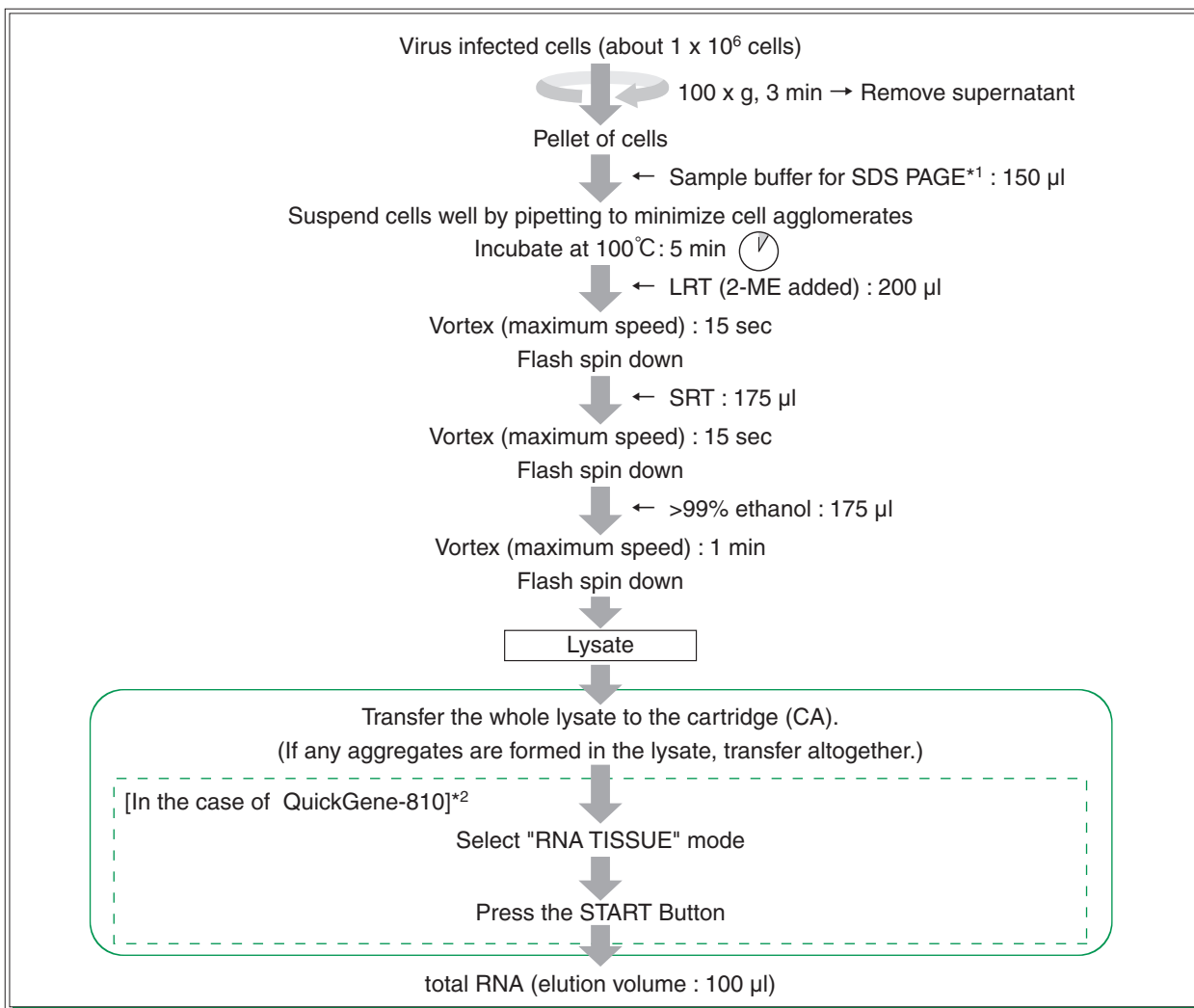
total RNA Extraction from SARS Coronavirus (SARS-CoV) infected Cells

Kit : QuickGene RNA tissue kit S II
Model : QuickGene-810 / QuickGene-Mini80

Summary

Enables easy and rapid extraction of RNA from SARS coronavirus (SARS-CoV) infected cells

● Protocol



*1 : Composition of sample buffer : 0.125 M Tris-HCl (pH 6.8), 10% (v/v) 2-mercaptoethanol, 4% (w/v) SDS, 10% (v/v) glycerol, 0.01% (w/v) bromophenol blue.

*2 : In the case of QuickGene-Mini80, please refer to the Kit Handbook for details.

* It is necessary to handle SARS coronavirus by P3. However, deactivation by present treatment makes it possible to handle it in common laboratories.

* Perform extraction within 30 min after lysate preparation.

Results : total RNA extraction from SARS coronavirus (SARS-CoV) infected cells

total RNA was extracted from SARS coronavirus (SARS-CoV) infected cells using QuickGene system (QuickGene and QuickGene RNA tissue kit S II) and Spin column method (A company). After 24 hour culture of SARS-CoV transmitted to Caco-2 cells , pellet of harvested cells was used as sample.

Cells No.1 : Noninfected Caco-2 cell
No.2 : SARS-CoV infected Caco-2 cell

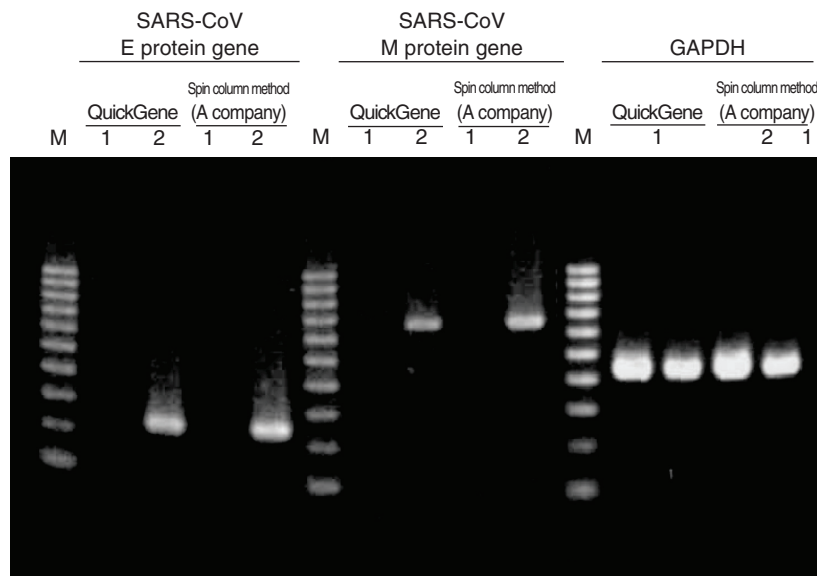
● The yield and purity of total RNA

Sample	Yield		Purity(A260/280)	
	No.1	No.2	No.1	No.2
QuickGene	9.4 µg	7.1 µg	1.93	1.90
Spin column method (A company)	7.6 µg	7.8 µg	1.80	1.88

The use of QuickGene system enables high-purity total RNA extraction with little contamination of protein in the almost same yield as Spin column method.

● RT-PCR

RT-PCR was performed with primer characteristic to E protein gene and M protein gene of SARS-CoV, GAPDH gene for total RNA extracted from SARS-CoV infected cells using QuickGene system and Spin column method (A company).



Electrophoresis condition : 2% agarose/ 1 x TAE

M : 100 bp DNA Ladder
1 : No.1 Noninfected Caco-2 cell
2 : No.2 SARS-CoV infected Caco-2 cell

RT-PCR products of E protein gene and M protein gene of SARS-CoV were detected for each total RNA of SARS-CoV infected cell.

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