

QuickGene QuickGene Series **Application Guide**

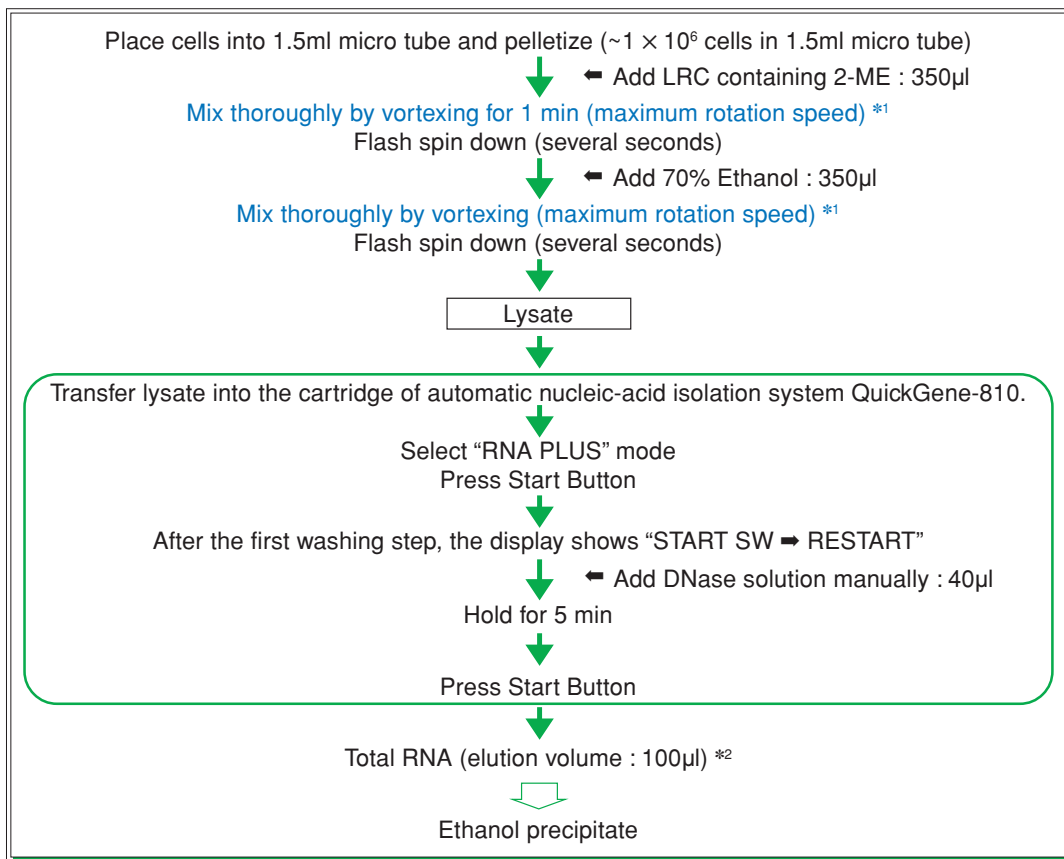
Viral RNA Isolation from Simian Immunodeficiency Virus (SIV) Infected Cells
QuickGene RNA Cultured Cell Kit S

Features

- Simultaneously extracts total RNA from 8 sets of Lysate in only 13 minutes
- Sophisticate total RNA isolation system without centrifugation
- Safety operation without using hazardous solvent such as phenol
- Isolated total RNA should be sufficiently pure and the yield is enough for RT-PCR, Northern Blotting analysis and other experiments because of uncontaminated protein and chaotropic salt.

Protocol

● **Total RNA isolation from Simian Immunodeficiency Virus (SIV) infected cells**



*1 : Mix completely by vortexing at the maximum speed.
If the mixing is not enough by vortexing, use tapping, pipetting or inverting.

*2 : This elution volume is initial value of "RNA PLUS" mode.

Results : Total RNA Isolation from SIV infected cells

Experiment 1: 10^6 CEM \times 174 cells were infected with SIV clone 1 (p27, corresponds to 67 ng) or SIV clone 2 (p27, corresponds to 75 ng). The cells were harvested after 96 hours culture then total RNA was isolated from the cells by using QuickGene-810 system (QuickGene-810 and the QuickGene RNA cultured cell kit S).

Experiment 2: 4×10^6 CEM \times 174 cells were infected with SIV clone 2 (p27, corresponds to 90 ng). The cells were harvested after 96 hours culture then total RNA was isolated from the cells by using QuickGene-810 system or spin column method.

● The yield of total RNA (μ g) (Sample: 1×10^6 SIV infected cells, with DNase treatment)

Virus	Experiment 1			Experiment 2			
	mock	SIV clone 1	SIV clone 2	mock	SIV clone 2	mock	SIV clone 2
QuickGene-810	5.6	3.8	7.0	8.0	3.6	6.0	9.5
Spin column	-	-	-	7.1	0.8	4.5	4.7

The yield of total RNA isolated with QuickGene-810 is higher than spin column method.

● The purity of total RNA (determination of protein contamination) : $A_{260/280}$ (with DNase treatment)

Virus	Experiment 1			Experiment 2			
	mock	SIV clone 1	SIV clone 2	mock	SIV clone 2	mock	SIV clone 2
QuickGene-810	1.86	1.82	1.84	1.90	1.86	1.77	1.91
Spin column	-	-	-	1.92	1.66	1.82	1.88

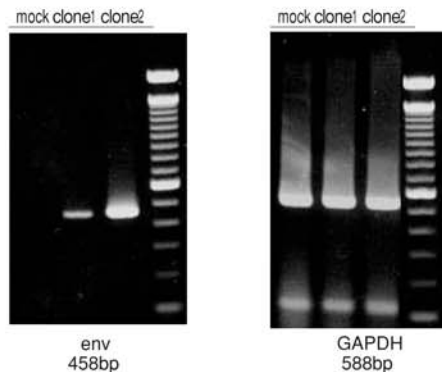
$A_{260/280}$: The ratio indicates the quality of nucleic acid from protein contamination.
(Protein contamination decreases the ratio.)

Compared to the spin column method, higher purity of total RNA with little protein contamination is achieved by using QuickGene-810 system.

● RT-PCR

AGE of RT-PCR with SIV-RNA isolated from SIV clone 1 or SIV clone 2 infected cells.

Experiment 1: SIV-RNA detection from SIV clone 1 or SIV clone 2 infected cells.



RT-PCR was performed on 1 μ g of isolated total RNA

RT-PCR amplification was performed successfully using total RNA.

As SIV clone 2 has higher infectiousness than SIV clone 1, larger amount of SIV-RNA can be isolated from SIV clone 2 infected cells.

Experiment 2: Comparison between QuickGene-810 and spin column



F: QuickGene-810
A: Spin column

Isolated S2V-RNA was used for RT-PCR template to amplify env and GAPDH gene.