Cat. # 7246

For Research Use

# TakaRa

## Human Mitochondrial DNA (mtDNA) Monitoring Primer Set

Product Manual

v201903Da

### **Table of Contents**

Ι.	Description	3
II.	Components	3
III.	Storage	3
IV.	Materials Required but not Provided	3
V.	Protocol	4
VI.	Experimental Example	7
VII.	Troubleshooting	8
VIII.	Reference	8
IX.	Related Products	8

#### I. Description

Human Mitochondrial DNA (mtDNA) Monitoring Primer Set is designed to quantify the relative number of copies of human mitochondrial DNA (mtDNA) using nuclear DNA (nDNA) content as a standard by real-time PCR. This set contains primer pairs for the amplification of four regions: two primer pairs for the detection of mtDNA and two primer pairs for the detection of nDNA. Each primer is specifically designed and will not amplify pseudogenes. As the primers are human-specific and will not amplify DNA from mice, human mtDNA content can be monitored in iPS cells or other cells cultured with feeder cells of mouse origin.

Using the four primer pairs in this set for real-time PCR, relative quantification of mtDNA is determined from the difference in Ct values for mtDNA and nDNA. This product provides a simple method for monitoring changes in the mtDNA copy number as mtDNA has been reported to increase during differentiation of pluripotent cells, such as iPS cells, into hepatic cells.

The primer pairs in this set are compatible with TB Green<sup>®</sup> *Premix Ex Taq*<sup>™</sup> II (Tli RNaseH Plus) (Cat. #RR820A/B).

#### II. Components (50 Reactions)

1.	ND1 Primer Mix (10 $\mu$ M each)*1	50 µ
2.	SLCO2B1 Primer Mix (10 $\mu$ M each)*2	50 u

- 3. ND5 Primer Mix (10  $\mu$  M each)\*1
- 4. SERPINA1 Primer Mix (10  $\mu$  M each)\*2 50  $\mu$ I
  - \*1 Primer for mitochondrial DNA (mtDNA)
  - \*2 Primer for nuclear DNA (nDNA)

#### III. Storage -20°C

- 1 year from date of receipt under proper storage conditions.
- When using repeatedly within a short period of time (~1 month), store at 4°C. However, be careful not to introduce contamination, as the product does not contain any preservatives.

#### IV. Materials Required but not Provided

#### (Reagents)

- NucleoSpin Tissue (Cat. #740952.10/.50/.250)
- TB Green Premix Ex Taq II (Tli RNaseH Plus) (Cat. #RR820A/B)\*3
- \* 3 We have begun the process of changing the names for Takara Bio's intercalator-based real-time PCR (qPCR) products to the "TB Green series". These products can be used the same way as before, as only the names are changing. Catalog number and product performance are unaffected by this transition.

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Cat. #7246

v201903Da

50 µl

(Instruments)

- Real-time PCR amplification device<sup>\*1</sup> Thermal Cycler Dice<sup>™</sup> Real Time System III (Cat. #TP950/TP970/TP980)<sup>\*2</sup> Thermal Cycler Dice Real Time System // (Cat. #TP900/TP960)<sup>\*2</sup> Thermal Cycler Dice Real Time System *Lite* (Cat. #TP700/TP760)<sup>\*2</sup>
- Spectrophotometer

#### (Equipment)

- Micropipettes
- Micropipette tips (with hydrophobic filters)
- 0.2-ml 8-strip tube, individual Flat Caps (Cat. #NJ600)\*2
- 0.1 ml 8-strip tube & cap Set (Cat. #NJ903)\*2
- \*1 When the Applied Biosystems StepOnePlus Real-Time PCR System (Thermo Fisher Scientific) is used, it may be necessary to set the threshold manually to obtain a flat baseline.
- \*2 Not available in all geographic locations. Check for ability in your area.

#### V. Protocol

#### Note:

To ensure proper use of reagents and equipment other than this product, refer to the user manual for each product.

#### **Precautions:**

Because mitochondrial DNA (mtDNA) is present in higher copy number than nuclear DNA (nDNA) and is smaller in size, there is a high possibility of mtDNA contamination in samples. It is important to protect samples from sources of human DNA contamination.

Use preventative measures, such as cleaning the working area and equipment with DNA-OFF<sup>™</sup> (Cat. #9036) or another solution for the prevention of DNA contamination. Also pay careful attention during PCR setup; change gloves regularly and wipe pipettes with a cleaner such as ethanol or DNA-OFF. Additionally, using an ultraviolet sterilization cabinet for PCR setup is an effective means of preventing contamination from equipment, etc.

#### Workflow

Extract genomic DNA (including mtDNA) from target cells.

Prepare highly purified DNA using NucleoSpin Tissue, etc.

Prepare master mix for real-time PCR and dispense into PCR reaction tubes.

↓ ↓

Add Primer Mixes. ↓

Perform real-time PCR.

Real-time PCR analysis of two mtDNA genes and two nDNA genes  $\downarrow$ 

Analyze data.

Relative quantification of the copy number of mtDNA using nDNA as the standard

For analysis, the file mtDNA\_Copy\_Number\_Calculation.xls can be used to calculate mtDNA copy number using Microsoft Office Excel. The file is available for download from the product page on our website.

#### PCR Reaction

Four reactions are performed for each DNA sample (one reaction each of the four primer pairs).

Refer to the reaction composition given below to prepare a master mix for the number of tubes needed. The master mix should contain the genomic DNA template, but should not contain the Primer Mix. Dispense 24  $\mu$ l of the master mix into each tube and then add 1  $\mu$ l of Primer Mix. Preparation of the reaction mixture using this procedure minimizes the amount of variation due to pipetting errors in the amount of template dispensed and provide more reliable results.

#### [ When using the Thermal Cycler Dice Real Time System // ]

\* Operate according to the instrument user manual.

1. Prepare the reaction mixture below on ice.

<Per Reaction>

Reagent	Volume	Final Conc.
TB Green <i>Premix Ex Taq</i> II (Tli RNaseH Plus) (2X)	12.5 μl	1X
Each Primer Mix (10 $\mu$ M each)	1 µ l	0.4 $\mu$ M each
Genomic DNA sample (5 ng/ $\mu$ l)*	2 µl	
Sterile purified water	9.5 µl	
Total	25 µl	

 Use 1 - 100 ng of genomic DNA per reaction.
Use highly purified DNA prepared using a purification kit such as NucleoSpin Tissue.

After purification, measure the  $OD_{260}/OD_{280}$  ratio of the genomic DNA to confirm purity.

2. Start the reaction using the conditions indicated below.



<When using the TB Green *Premix Ex Taq* II (Tli RNaseH Plus)>

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3. The reaction is complete.

Perform data analysis and determine the mtDNA copy number by relative quantification.

Calculate the copy number of mtDNA from the Ct values obtained for each of the four target genes.

Follow the procedures below. The copy number of mtDNA is calculated as the average of the values calculated based on the combinations of the primers as indicated below.

**Note:** The Microsoft Office Excel file mtDNA\_Copy\_Number\_Calculation.xls\* can be used for the calculation of the mtDNA copy number by inputting the Ct values for each primer.

This file can be used to analyze up to 5 experimental replicates and will calculate the average mtDNA copy number.

#### Calculation:

- a. Determine the difference in the Ct values for the ND1/SLCO2B1 pair.  $(\Delta Ct1 = Ct \text{ for SLCO2B1} Ct \text{ for ND1})$
- b. Determine the difference in the Ct values for the ND5/SERPINA1 pair in the same manner. ( $\Delta$  Ct2 = Ct for SERPINA1- Ct for ND5)
- c. Find  $2^{\Delta Ct}$  for the values for  $\Delta Ct1$  and  $\Delta Ct2$ .
- d. Use the average of the 2 values found in step c. as mtDNA copy number.

Example of the Calculation of Copy Number

Primer	Ct Value	∆Ct	2∆Ct	Mean Copy Number of mtDNA
ND1	15.75	Λ C+1 - 8 78	$\Delta Ct1 = 8.78$ 440	574
SLCO2B1	24.53	ΔCT = 0.76		
ND5	15.04	$\Lambda$ (+2 - 0.47	700	574
SERPINA1	24.51		709	

\* Download from the product page on our website.

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#### VI. Experimental Example

Relative quantification of the copy number of mtDNA in two types of human iPS cells and liver tissue was performed using genomic DNA templates (including mtDNA).

(Method)

Detection of mtDNA was carried out according to the protocol. Refer to "V. Protocol" for the reaction conditions and the method for calculating the copy number of mtDNA.

(Results)

The copy number of mtDNA has been reported to increase during differentiation of pluripotent cells, such as iPS cells, into hepatic cells. This experiment demonstrated that the mtDNA copy number in liver tissue, which is mostly comprised of hepatic cells, is greater than that of pluripotent cells.

Sample	Primer	Ct Value	∆Ct	2 <sup>∆Ct</sup>	Mean copy number	
	ND1	17.19	7.17	144	- 231	
iDS coll 1	SLCO2B1	24.36				
IPS Cell-1	ND5	16.17	8.31	217		
	SERPINA1	24.48		517		
	ND1	17.08	7.37	7.27	165	
	SLCO2B1	24.45		105	260	
IPS Cell-2	ND5	16.19	8.47	355		
	SERPINA1	24.66				
	ND1	15.75	8.78	440	574	
Human	SLCO2B1	24.53		440		
liver tissue	ND5	15.04	9.47	700	5/4	
	SERPINA1	24.51		9.47	709	



Comparison of the mtDNA Copy Number

#### VII. Troubleshooting

If no amplification is observed during real-time RT-PCR

- The quantity of mtDNA may be unusually small due to the tissue, strain, and culture conditions of the cells. Optimize the quantity of template DNA used within the range of 1 100 ng as necessary.
- The use of genomic DNA (including mtDNA) of extremely low purity is not recommended. Use DNA that has been isolated using a purification kit such as NucleoSpin Tissue.
- Perform DNA extraction using the same method for all samples. Preparing using a different method may cause great differences in purity and affect the results.
- Prepare the real-time PCR reaction solution on ice and perform PCR as soon as possible. When on leaving the assembled reaction on ice, shield from light until the start of the reaction.

#### VIII. Reference

- 1) Yue Yu, *et al.* Hepatocyte-like cells differentiated from human induced pluripotent stem cells: Relevance to cellular therapies. *Stem Cell Research*. (2012) **9**: 196-207.
- 2) Xu, Xiuling, *et al*. Mitochondrial regulation in pluripotent stem cells. *Cell metabolism*. (2013)**18**.3: 325-332.

#### **IX.** Related Products

NucleoSpin Tissue (Cat. #740952.10/.50/.250) NucleoSpin Tissue XS (Cat. #740901.10/.50/.250) TB Green<sup>®</sup> *Premix Ex Taq*<sup>™</sup> II (Tli RNaseH Plus) (Cat. #RR820A/B) Thermal Cycler Dice<sup>™</sup> Real Time System III (Cat. #TP950/TP970/TP980)\* Thermal Cycler Dice<sup>™</sup> Real Time System *Lite* (Cat. #TP900/TP960)\* Thermal Cycler Dice<sup>™</sup> Real Time System *Lite* (Cat. #TP700/TP760)\* 0.1 ml 8-strip tube & cap Set (Cat. #NJ903)\* 0.2 ml 8-strip tube, individual Flat Caps (Cat. #NJ600)\* 0.1 ml 96 well plate for Real Time (Cat. #NJ900)\* 96well Hi-Plate for Real Time (Cat. #NJ400)\* Sealing Film for Real Time (Cat. #NJ500)\* DNA-OFF<sup>™</sup> (Solution for elimination of DNA contamination) (Cat. #9036)

\* Not available in all geographic locations. Check for availability in your area.

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