AccuPower® qPCR Array System: Human Immune Checkpoint qPCR Panel Kit

(V0/2021-10-12)

[Cat. No.] S-6042-PH3

Introduction

BIONEER offers 39 Human Immune Checkpoint relevant genes, 6 reference genes and 3 control primers. Human Immune checkpoint qPCR panel kit is easy-to-use product as it simplifies preparation of real-time PCR mixture by making the user add the template DNA, 2X Master Mix (hydrolysis probe type), and nuclease-free water only. All primers and probes are designed and validated in accordance with the MIQE (Minimum Information for Publication of Quantitative Real-Time PCR Experiments) guidelines*.

* Bustin, S.A., et al. 2009. The MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments, Clinical Chemistry 55:4, 611-622.

Features & Benefits

- Fidelity: qPCR primer and probe with qPCR efficiency of 90-110%, with guaranteed detection limit of 100 copies.
- User-friendly: Simplified procedure starting just by adding the template and Master Mix you want to analyze.
- Economic: Reduced time and cost of primer and probe design, synthesis and efficiency assays.

Components

Components	Amount
Human Immune Checkpoint qPCR Panel Kit (96 well plate)	1 plate
Adhesive Optical Sealing Film (Cat.No. 3111-4110)	1 sheet per plate

Storage

- This product is shipped at ambient temperature.
- Store at room temperature. If stored in the recommended temperature, this product will be stable for 2 years after the delivery date.

Online Resources

Korean **English**

Visit our **product page** for additional information and protocols

Ordering Information

Description		Cat. No.
AccuPower® qPCR Array System: Human Immune Checkpoint qPCR Panel Kit	48 genes	S-6042-PH3

Notice

BIONEER corporation reserves the right to make corrections, modifications, improvements and other changes to its products, services, specifications or product descriptions at any time without notice.

Explanation of Symbols

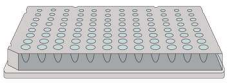
Plate Map

	1	2	3	4	5	6	7	8	9	10	11	12
A	PDCD1	TNFRSF4	CD160	TIGIT	CD47	B2M	PDCD1	TNFRSF4	CD160	TIGIT	CD47	B2M
B	CD80	CD27	TNFRSF9	CD226	CD70	GAPDH	CD80	CD27	TNFRSF9	CD226	CD70	GAPDH
C	CD86	CD40	TNFSF9	PVR	CD276	GUSB	CD86	CD40	TNFSF9	PVR	CD276	GUSB
D	CD28	CD40LG	ICOSLG	CD274	CSF1R	HPRT1	CD28	CD40LG	ICOSLG	CD274	CSF1R	HPRT1
E	CTLA4	CSF1	ICOS	PDCD1LG2	CEACAM1	RPLP0	CTLA4	CSF1	ICOS	PDCD1LG2	CEACAM1	RPLP0
F	TNFRSF14	IDO1	VSIR	LGALS1	LAG3	NTC	TNFRSF14	IDO1	VSIR	LGALS1	LAG3	NTC
G	BTLA	VTCN1	TNFRSF18	LGALS3	HAVCR2	GDC	BTLA	VTCN1	TNFRSF18	LGALS3	HAVCR2	GDC
H	TNFSF4	TNFSF14	TNFSF18	LGALS9	ACTB	PPC	TNFSF4	TNFSF14	TNFSF18	LGALS9	ACTB	PPC

Table 1. Layout of *AccuPower*® qPCR Array System: Human Immune checkpoint qPCR panel kit

The panel is involved 39 target genes requested (A1-G5, A7-G11), 6 reference genes (H5-E6, H11-E12) and 3 control primers (F6-H6, F12-H12).

Experimental Procedures

<table>
<tr><th colspan="2">Steps</th><th>Procedure Details</th></tr>
<tr><td>1</td><td> 
Preparation of reaction mixture</td><td>1. Prepare template DNA, AccuPower® Plus DualStar™ qPCR Master Mix (K-6603, not provided), and nuclease-free water in a tube to make a total volume of 50 µl as described in following table.
<table>
<tr><th>Components</th><th>50 µl reaction</th></tr>
<tr><td>AccuPower® Plus DualStar™ qPCR Master Mix</td><td>25 µl</td></tr>
<tr><td>Template DNA</td><td>5 pg-100 ng</td></tr>
<tr><td>Nuclease-free water</td><td>Variable</td></tr>
<tr><td>Total volume</td><td>50 µl</td></tr>
</table></td></tr>
<tr><td>2</td><td> 
Resuspension of primers</td><td>2. Carefully remove the covered film of panel and dispense 50 µl of reaction mixture into each well of AccuPower® qPCR Array System: Human Immune checkpoint qPCR panel kit.
* Note: Change pipette tips following each pipetting step to avoid cross-contamination among the wells.

3. Seal the plate with adhesive optical sealing film and briefly spin down.

4. Then, completely mix by vortexing to resuspend of lyophilized primers and spin down again.
* Note: Before start, check carefully if there are residues on the film.</td></tr>
<tr><td>3</td><td> 
Real-time PCR</td><td>5. Perform the reaction under the following conditions.
<table>
<tr><th>Step</th><th>Temperature</th><th>Time</th><th>Cycles</th></tr>
<tr><td>Pre-denaturation</td><td>95°C</td><td>5 min</td><td>1 cycle</td></tr>
<tr><td>Denaturation</td><td>95°C</td><td>5 sec</td><td></td></tr>
<tr><td>Annealing/Extension</td><td>58°C</td><td>15 sec</td><td>45 cycles</td></tr>
<tr><td>Detection</td><td colspan="2">Scan</td><td></td></tr>
<tr><td>Melting</td><td>20°C</td><td>1 min</td><td>1 cycle</td></tr>
</table>
6. After the reaction, perform data analysis.</td></tr>
<tr><td></td><td> 
Control primers</td><td>1) Non Template Control (NTC)
- NTC is a negative control for checking on random or on reagent contamination.
- Just add the pre-mixture containing nuclease-free water but excluding the template into NTC well.
- If the value of Ct^{NTC} is less than 35, there is overall DNA contamination in your PCR system. In this case, clean up the equipment and replace all the reagents to new ones.

2) Genomic DNA Control (GDC)
- GDC primer is for the detection of non-transcribed genomic DNA contamination.
- In GDC well, primers which target genomic DNA are coated.
- Add pre-mixture (your template, 2X Master Mix and nuclease-free water) into the GDC well.
- If the value of Ct^{GDC} is less than 35, gDNA contamination might have occurred in your RNA samples. In this case, you ought to conduct an additional DNase treatment to clean up your samples.

3) Positive PCR Control (PPC)
- PPC primer is for the PCR test.
- The PPC well contains positive template and primers, so just add 2X Master Mix and nuclease-free water into the PPC well.
- The value of Ct^{PPC} should be referred to the quick manual.</td></tr>
</table>