COSMO BIO CO., LTD.
Inspiration for Life Science

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Human Tri-Lineage Multiplex PCR Kit

Store at -20°C

Cat. No.	Description	Quantity
G286	Human Tri-Lineage Multiplex PCR Kit	100 reactions

Product Description

The Human Tri-Lineage Multiplex PCR Kit is intended for use in characterizing human embryonic stem cells (hESCs) and pluripotent human embryonal carcinoma stem cells (hECs). It allows for the simultaneous determination of stem cell pluripotency and differentiation state through a multiplex PCR reaction. This method of characterization is fast, effective, and has less stringent sample volume requirements compared to methods such as immunocytochemistry.

Kit Components

Component Name	Volume	Part No.
2X PCR Taq Mastermix	1.25ml X 2	G286-1
Tri-Lineage Primer Mix	100µl	G286-2

Shipping and Storage

This product is stored at -20°C. Avoid freeze-thaw cycles by making aliquots upon first thawing.

Protocol Overview

This kit uses cDNA that has been reverse transcribed from purified sample total RNA. Five sets of primers are simultaneously amplified in a multiplex PCR reaction. When the results are visualized using agarose gel electrophoresis, the presence or absence of PCR products relating to each primer will provide information pertaining to the differentiation state and pluripotency of the source cells. The following five sets of primers are used:

Primer	Fragment Size	Purpose
Pou5f1/Oct4	~500bp	Indicates active pluripotent state
AFP	~400bp	Indicates differentiation into endoderm lineage
ACTC1	~300bp	Indicates differentiation into mesoderm lineage
SOX1	~200bp	Indicates differentiation into ectoderm lineage
GAPDH	~1kb	Internal standard for normalizing RNA concentration

Protocol

1. Set up the multiplex PCR reaction according to the table below:

Component	Test sample	NTC Control
2X PCR Taq Mastermix	25µl	25µl
Tri-Lineage Primer Mix	1µl	1µl
cDNA from sample	1µl	-
ddH_2O	23µl	24µl
Total volume per reaction	50µl	50µl

2. Perform 30-40 cycles of PCR amplification as follows:

Step	Temperature	Duration	Cycles
Enzyme activation	95°C	5min	Hold
Denature	95°C	30sec	30-40
Anneal	60°C	30sec	
Extend	72°C	60sec	
Final extension	72°C	10min	1
Holding	4°C	-	Hold

3. Analyze the amplification products by agarose gel electrophoresis and visualize by ethidium bromide or SafeView™ (Cat. No. G108) staining. Since the PCR Taq Mastermix already contains a loading dye, further addition of loading dye is not required. For DNA fragment length confirmation, the 1kb Plus OptiDNA Marker (Cat. No. G248) is recommended.

4. The presence of bands at the pre-specified fragment sizes (see table on previous page) will indicate the pluripotency, the differentiation state, as well as the RNA content of the stem cells analyzed.

Recommendations for Optimal Result

- The 2X PCR Taq Mastermix included in this kit is optimized for multiplex PCR. Please do not employ any PCR reagents other than the materials in this kit.
- Make aliquots of the reagents to avoid contamination.
- Start the PCR as soon as the reaction mixture is prepared and always keep the reaction mixture chilled on ice prior to PCR reactions.