hPSC Genetic Analysis Kit

qPCR analysis kit for detecting the majority of karyotypic abnormalities reported in human ES and iPS cells

Catalog #07550 60 Reactions

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Product Description

hPSC Genetic Analysis Kit contains nine primer-probe mixes to detect the majority of recurrent karyotypic abnormalities reported in human embryonic stem (ES) cells and induced pluripotent stem (iPS) cells. This qPCR-based kit enables the genetic screening of multiple human ES and iPS cell lines in a rapid and cost-effective manner. It uses double-quenched probes with a 5-carboxyfluorescein (5-FAM) dye to give superior performance over other single-quenched probes.

hPSC Genetic Analysis Kit contains a Genomic DNA Control sample that has been validated as a diploid control for the regions analyzed using this kit, as well as a separate ROX Reference Dye. The kit contains sufficient material to analyze 20 individual samples in triplicate (60 reactions [rxn]).

Product Information

All components listed below are stable until expiry date (EXP) on label.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE
qPCR Master Mix (2X)	07551	3 mL	Store at -20°C.
ROX Reference Dye	07552	0.2 mL	Store at -20°C.
Chr 1q Genetic Assay	07553	60 Rxn	Store at -20°C.
Chr 4p Genetic Assay	07554	60 Rxn	Store at -20°C.
Chr 8q Genetic Assay	07555	60 Rxn	Store at -20°C.
Chr 10p Genetic Assay	07556	60 Rxn	Store at -20°C.
Chr 12p Genetic Assay	07557	60 Rxn	Store at -20°C.
Chr 17q Genetic Assay	07558	60 Rxn	Store at -20°C.
Chr 18q Genetic Assay	07559	60 Rxn	Store at -20°C.
Chr 20q Genetic Assay	07560	60 Rxn	Store at -20°C.
Chr Xp Genetic Assay	07561	60 Rxn	Store at -20°C.
Genomic DNA Control	07562	15 µL	Store at -20°C.
TE Buffer	07563	1 mL	Store at -20°C.

Materials Required But Not Included

PRODUCT NAME	CATALOG #
Genomic DNA extraction kit	e.g. QIAGEN 69504
Costar® Microcentrifuge Tubes, 0.65 mL	38037
384-well qPCR plate	e.g. Sigma Z374911
Nuclease-free water	e.g. Sigma W4502
Optical adhesive film	e.g. Sigma Z707465

Preparation of Reagents and Materials

A. Harvesting Genomic DNA

Harvest genomic DNA from the cell line to be analyzed using an appropriate genomic DNA extraction kit. If not used immediately, store harvested DNA at -20°C.

B. Master Mix + Dye

1. Thaw qPCR Master Mix (2X) and ROX Reference Dye on ice. Protect from light.
2. Add ROX Reference Dye to qPCR Master Mix (2X) according to Tables 1 and 2. For instruments not listed, refer to the manufacturer's instructions.

Table 1. Recommended Reference Dye Concentration Levels for PCR Systems

PCR SYSTEM	REFERENCE DYE CONCENTRATION LEVEL		
	HIGH	LOW	NONE
Applied Biosystems • 7900HT Fast and 7300 Real-Time PCR Systems • StepOne™ and StepOnePlus™ Real-Time PCR Systems	X		
Applied Biosystems • ViiA™ 7 and 7500 Real-Time PCR Systems • QuantStudio™ Flex		X	
Agilent Technologies • Mx3005P and Mx4000P		X	
Bio-Rad • CFX, iQ™, and DNA Engine Opticon® Real Time PCR Systems			X
Roche • LightCycler® Real-Time PCR System			X

Table 2. Volume of ROX Reference Dye to Add to Master Mix

VOLUME OF MASTER MIX	VOLUME OF ROX REFERENCE DYE	
	High Reference Dye System (see Table 1)	Low Reference Dye System (see Table 1)
3 mL	120 µL	12 µL

3. Pulse vortex Master Mix + Dye on high for 3 - 5 seconds. Place on ice and protect from light.

   NOTE: If not used immediately, aliquot and store at -20°C. Do not exceed the expiry date on the label. After thawing the aliquots, use immediately. Do not re-freeze.

C. Genomic DNA Control and Samples

1. Thaw Genomic DNA Control and samples on ice.
2. Measure the concentration of the genomic DNA samples to be analyzed using a NanoDrop™ spectrophotometer or other method.

   NOTE: At least two samples (or one sample plus the control) are required to determine copy number.
3. In a 0.65 mL microcentrifuge tube, add 1.5 µL of Genomic DNA Control to 56.5 µL of nuclease-free water (final concentration 5 ng/µL). Vortex on high for 3 - 5 seconds. Place on ice.
4. Add 290 ng of genomic DNA sample to separate 0.65 mL microcentrifuge tubes and adjust the volume to 58 µL with nuclease-free water (final concentration 5 ng/µL). Place tubes on ice.

   NOTE: If the concentration of genomic DNA samples is > 290 ng/ µL, it is recommended to dilute the sample using nuclease-free water so that an appropriate volume of genomic DNA is added (> 1 µL).
5. Vortex Master Mix + Dye (prepared in section B) on high for 5 seconds. Add 145 µL to each genomic DNA sample and the control (prepared in steps 3 - 4). Pipette up and down 2 - 3 times to mix. Place the tubes on ice and protect from light.

   NOTE: Master Mix + Dye is viscous; pipette with care to avoid loss of material in the pipette tip.

D. Primer-Probe Mixes

1. Prepare the primer-probe stock solutions as follows:
   a. Centrifuge the primer-probe sequences (e.g. Chr 4p Genetic Assay) at 750 x *g* for 10 seconds to ensure the contents are at the bottom of the tube.
   b. Add 33 µL of TE Buffer to each tube. Pipette the solution up and down the sides of the tube to ensure complete resuspension.

      NOTE: Use a separate pipette tip between Genetic Assays to avoid cross-contamination.
   c. Centrifuge tubes at 750 x *g* for 10 seconds.

      NOTE: If not used immediately, aliquot and store at -20°C. Do not exceed the expiry date on the label. After thawing the aliquots, use immediately. Do not re-freeze.
2. Calculate the number of reactions required for each primer-probe using the equation below:

   Number of reactions per primer-probe = (Number of genomic DNA samples [including control] to be analyzed x 3) + n

   Where:

   - For 2 - 5 samples, n = 2 OR
   - For 6 - 10 samples, n = 3 OR
   - For > 10 samples, n = 10% of number of samples

3. Prepare the primer-probe mixes by combining each primer-probe stock solution (prepared in step 1) with nuclease-free water in individual 0.65 mL microcentrifuge tubes. Refer to Table 3 for example volumes or calculate the volumes required as follows:

   Volume of nuclease-free water = Number of reactions per primer-probe x 2.5 µL

   Volume of primer-probe stock solution = Number of reactions per primer-probe x 0.5 µL

   Vortex each tube on high for 5 seconds.

Table 3. Example Volumes for Preparing Primer-Probe Mixes

NUMBER OF SAMPLES	2	3	4	5	6	7	8	9	10
NUMBER OF REACTIONS	8	11	14	17	21	24	27	30	33
VOLUME OF NUCLEASE-FREE WATER (µL)	20	27.5	35	42.5	52.5	60	67.5	75	82.5
VOLUME OF PRIMER-PROBE STOCK SOLUTION (µL)	4	5.5	7	8.5	10.5	12	13.5	15	16.5
TOTAL VOLUME (µL)	**24**	**33**	**42**	**51**	**63**	**72**	**81**	**90**	**99**

4. Place the tubes on ice and protect from light.

Directions for Use

Please read the entire protocol before proceeding.

A. qPCR

1. Using a felt pen, mark a 3 x 9-well boundary for each sample on a 384-well PCR plate according to Figure 1 or Figure 2, depending on the number of samples. Tape the top edge of the plate to a microcentrifuge tube rack (or similar) to create an ~30° angle.

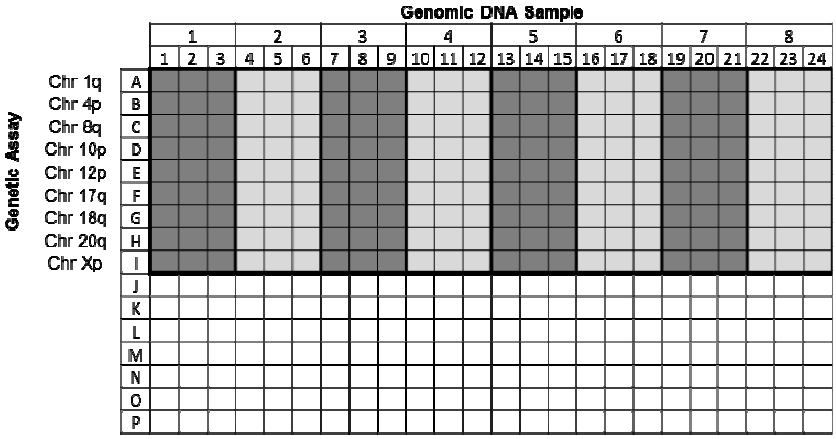

Figure 1. PCR Plate Array for up to 8 Samples

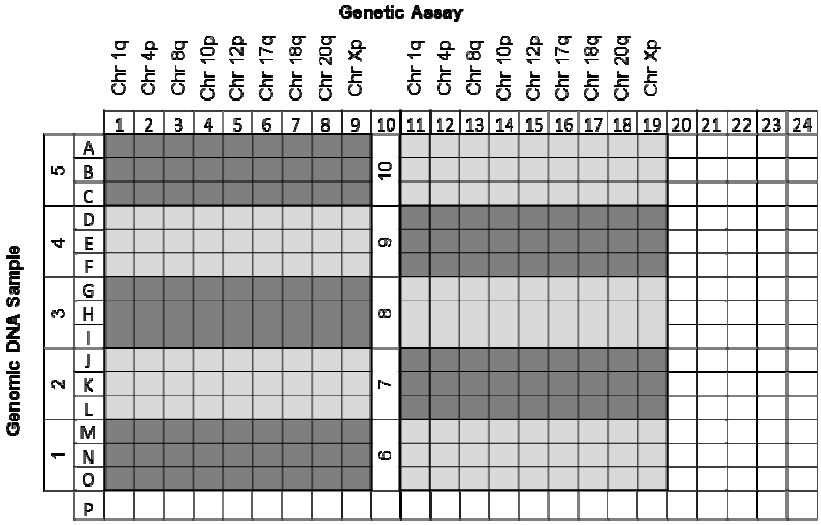

Figure 2. PCR Plate Array for 10 Samples

2. For the control and each genomic DNA sample prepared in section C (containing Master Mix + Dye), vortex on high for 5 seconds then centrifuge briefly to collect material at the bottom of the tube. Add 7 µL to the bottom edge of each well of the prepared plate, keeping within the marked 3 x 9-well boundary. Repeat for each sample until all samples have been loaded.
3. Working with one primer-probe mix at a time, vortex on high for 5 seconds, then centrifuge briefly to collect material at the bottom of the tube. Add 3 µL to the appropriate row of the plate, at the top edge of the well. Repeat until all primer-probe mixes have been loaded.
4. Cover the plate using optical adhesive film; use a scraper or roller to ensure that all edges and wells are sealed. Centrifuge the plate at 1000 x *g* for 1 - 2 minutes in a swinging bucket rotor fitted with plate holders to collect material at the bottom of the well.
5. Using a quantitative PCR system run the plate using the cycling conditions indicated in Table 4.

Table 4. PCR Cycling Conditions

STAGE	CYCLES	TEMPERATURE (°C)	FAST CYCLING TIME (min:sec)	STANDARD CYCLING TIME (min:sec)
Polymerase activation	1	95.0	03:00	03:00
Amplification:	40			
Denature		95.0	0:05	0:15
Anneal/extend		60.0	0:30	1:00

B. ANALYSIS OF RESULTS

Results obtained using hPSC Genetic Analysis Kit can be analyzed using the application available at www.stemcell.com/geneticanalysisapp.

NOTE: Organize the Ct values (also referred to as Cq values) into the format shown in Figure 3; the data can then be pasted directly into the table in the application.

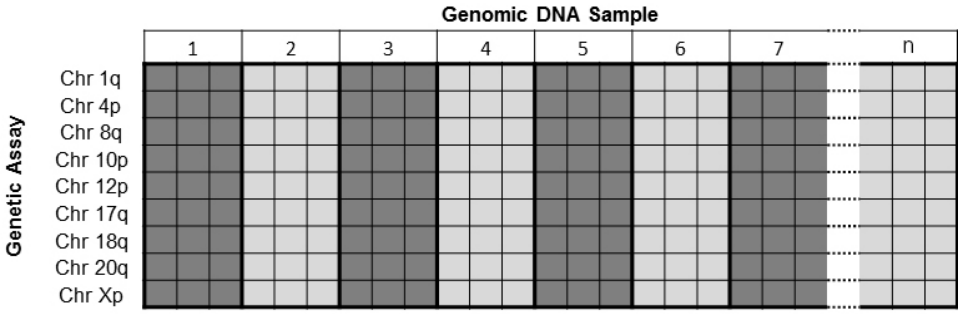

Figure 3. Data Organization for Analysis

Alternatively, data can be analyzed using the methods described below.

1. Calculate ΔCt values as follows:

   Subtract the replicate Ct values of each Genetic Assay from the average Ct value of Chr 4p Genetic Assay.

   NOTE: This calculation normalizes the data within each sample to account for any differences in DNA concentration between samples.

2. Calculate ΔΔCt values as follows:

   Subtract the replicate Ct values of each test sample from the average Ct value of the Genomic DNA Control sample for each Genetic Assay.

   NOTE: This calculation normalizes the data to a known control sample and will be the basis of determining copy number.

3. Calculate copy number as follows:

   Copy number = $(2^{-\Delta\Delta Ct}) \times 2$

   NOTE: A copy number < 1.8 or > 2.2 with a p-value*< 0.05 may indicate the presence of an abnormality within the culture.

   NOTE: High variability between technical replicates will significantly impact downstream analysis. This is particularly important in any Genetic Assay within the control sample and also for the Chr 4p Genetic Assay within test samples, as this data is used for normalization.

   *p-values can be calculated across all loci using a one-way ANOVA with a tukey post-hoc test. Alternatively, an unpaired t-test can be performed between the Chr 4p Genetic Assay control region and the locus of interest.