Rev.0

HeLa Cell Contamination Detection PCR Kit (HLCC)

Catalog #8988
100 reactions

Product Description

HeLa cells were originally derived from a highly aggressive cervical cancer cell in the early 1950s. As the first immortal human cell line cultured, HeLa cells are now one of the most popular cell lines used by researchers around the world. HeLa cells can easily propagate many generations in culture and if HeLa cells contaminate other cells, they can quickly outgrow the original cells. As a result, HeLa cell contamination has become a significant issue for the scientific community. Due to the high potential for HeLa cell contamination in cell lines, a routine assessment is recommended.

ScienCell's HeLa Cell Contamination Detection PCR Kit (HLCC) is designed for sensitive, fast and easy detection of HeLa cell contamination in cultured human cells by PCR. HLCC can utilize either cell lysate or purified genomic DNA as the PCR template. The cell lysis buffer and enhancer (Cat #GQ400a and GQ400b) are included in the kit to lyse pelleted cell samples. The HeLa genomic DNA (gDNA) detection primer set (HLD) recognizes and amplifies a HeLa cell-specific sequence in the genome that is not present in other human cells. The human gDNA control primer set (HGC) recognizes and amplifies a common genomic region shared by all human cells including HeLa cells. The carefully designed primers are free of non-specific amplification using recommended PCR conditions. Each primer set has been validated by PCR and gel electrophoresis for amplification specificity. This highly sensitive kit can detect as low as 20 HeLa cells/million cells.

Kit Components

Cat #	Component	Quantity	Storage
MB6068-1	2x CitriNStart Taq PCR master mix, 1 mL	5 vials	-20°C
8988a	HeLa gDNA detection primer set (HLD), lyophilized	1 vial	-20°C
8988b	Human gDNA control primer set (HGC), lyophilized	1 vial	-20°C
GQ100-04	Nuclease-free H_2O	4 mL	4°C
GQ400a	Cell lysis buffer	10 mL	4°C
GQ400b	Cell lysis buffer enhancer, 100x	100 µL	-20°C
8988f	HeLa positive control PCR template	100 µL	-20°C

Additional Materials Required (Materials Not Included in Kit)

Component	Recommended
Cell pellets	Customers' samples
Heat blocks	with upper temperature limit above 95°C
PCR instrument	
PCR plates or tubes	PCR instrument-dependent
gDNA extraction kit ***(optional)***	SpeeDNA Isolation Kit (ScienCell, Cat #MB6918)

Quality Control
The specificity of the primer sets is validated by PCR using HeLa cells and more than 10 human primary cell types. The PCR products are analyzed by gel electrophoresis.

Product Use
HLCC is for research use only. It is not approved for human or animal use, or for application in clinical or *in vitro* diagnostic procedures.

Shipping and Storage
The product is shipped on dry ice. Upon receipt, store CitriNStart Taq PCR master mix (Cat #MB6068-1), primer set vials (Cat #8988a and #8988b), cell lysis buffer enhancer (Cat #GQ400b) and HeLa positive control PCR template (Cat #8988f) at -20°C in a manual defrost freezer, and nuclease-free H_2O (Cat #GQ100-04) and cell lysis buffer (Cat #GQ400a) at 4°C. Once CitriNStart Taq PCR master mix is thawed, store it at 4°C and do not refreeze. CitriNStart Taq PCR master mix is stable at 4°C for up to 6 months if stored properly.

Procedures

Important: ***Only*** *use polymerases with hot-start capability to prevent possible primer-dimer formation.* ***Only*** *use nuclease-free reagents in PCR amplification.*

1. Preparation of cell lysate samples

Note: Skip Section A if using purified genomic DNA as the PCR template.

1. For each sample, count cell numbers to be harvested. Harvesting 0.5-2 million cells/sample is recommended. Wash cells with PBS once, pellet cells and carefully remove PBS.

2. Determine the total volume of cell lysis buffer (Cat #GQ400a) to be used for the samples at 20,000 cells/µL cell lysis buffer. Transfer the calculated amount of cell lysis buffer with 5% extra to a new tube. Supplement the aliquoted cell lysis buffer with cell lysis buffer enhancer (100x, Cat #GQ400b). For every milliliter of cell lysis buffer, add 10 µL of cell lysis buffer enhancer (see an "example of calculations" below).

3. Transfer the supplemented cell lysis buffer to each cell pellet sample at 20,000 cells/µL supplemented cell lysis buffer. Carefully pipette the cell pellet up and down 20 times without generating bubbles. The samples should be homogenous. If not, continue pipetting until fully homogenized.

4. Incubate the homogenized samples at 55°C for 30 minutes, followed by incubating at 95°C for 10 minutes to fully lyse the samples. Alternatively, transfer 20 µL of each homogenized sample from step A.3 to a PCR tube, and run a PCR program as shown in Table 1.

Table 1. PCR program settings for lysing the cells

Step	Temperature	Time	Number of cycles
1	55°C	30 min	1
2	95°C	10 min	1
Hold	4°C	Indefinite	1

Example of calculations: Sample A has 0.5 million cells and sample B has 2.0 million cells.

In step **A.2**, aliquot (0.5 + 2.0) x 10^6/ 20,000 x 105% µL = 132 µL of cell lysis buffer (Cat #GQ400a), then add 132 µL x 10 µL/1 mL = 1.32 µL of cell lysis buffer enhancer (100x, Cat #GQ400b) to the aliquoted cell lysis buffer.

In step **A.3**, transfer 0.5 x 10^6/ 20,000 = 25 µL of supplemented cell lysis buffer to sample A, and 2.0 x 10^6/ 20,000 = 100 µL of supplemented cell lysis buffer to sample B.

5. Spin the samples at 1,000 rpm for 10 seconds at room temperature.

6. Keep lysed samples on ice or store at -20°C. Samples can be stored at -20°C for up to 12 months.

B. PCR setup

1. When using this kit for the first time, allow primer vials (Cat #8988a and #8988b) to warm to room temperature.

2. Centrifuge the vials at 1,500x g for 1 minute.

3. Add 200 µl nuclease-free H_2O (Cat #GQ100-04) to HLD primer set (lyophilized, Cat #8988a) to make HLD primer stock solution. Aliquot as needed. Store at -20°C in a manual defrost freezer. Avoid repeated freeze-and-thaw cycles.

4. Add 200 µl nuclease-free H_2O (Cat #GQ100-04) to HGC primer set (lyophilized, Cat #8988b) to make HGC primer stock solution. Aliquot as needed. Store at -20°C in a manual defrost freezer. Avoid repeated freeze-and-thaw cycles.

5. For each sample, prepare two PCR reactions, one with HLD primer stock solution, and one with HGC primer stock solution. Prepare 50 µl PCR reaction per well as shown in Table 2.

6. For HeLa Positive control PCR template (Cat #8988f), prepare two PCR reactions, one with HLD primer stock solution, and one with HGC primer stock solution. Prepare 50 µl PCR reaction per well as shown in Table 2.

7. Prepare two "no-lysate" negative control PCR reactions by using 5 µL of supplemented lysis buffer as the PCR template (no cell lysate added), one with HLD primer stock solution, and one with HGC primer stock solution. Prepare 50 µl PCR reaction per well as shown in Table 2.

   Note: When using purified genomic DNA as the template, dilute the gDNA to 80 ng/µL, and take 5 µL for each PCR reaction. For samples less than 80 ng/µL, 400 ng of gDNA should be prepared per well with less H_2O used to bring the total volume up to 50 µL.

Table 2. PCR reaction preparation

Template: lysed cell sample, gDNA sample, positive control (Cat #8988f), or lysis buffer	5 µl
Primer stock solution (HLD or HGC)	2 µl
CitriNStart Taq PCR master mix, 2x (Cat #MB6068-1)	25 µl
Nuclease-free H_2O (Cat #GQ100-04)	18 µl
Total volume	***50 µl***

8. Seal the PCR reaction wells. Centrifuge the PCR plates or tubes at 1,500x g for 15 seconds.

9. Refer to Table 3 for PCR program setup.

   Note: The primary factors that determine optimal annealing temperature are the primer length and primer composition. Based on the properties of HLD and HGC primer sets (Cat #8988a and #8988b), we highly recommend an annealing temperature of 63°C as shown in Table 3:

Table 3. Recommended PCR Program

Step	Temperature	Time	Number of cycles
Pre-incubation	95°C	10 min	1
Denaturation	95°C	20 sec	36
Annealing	63°C	20 sec	
Extension	72°C	20 sec	
Hold	20°C		

10. Analyze the PCR products by gel electrophoresis on a 1.5% agarose gel.

Appendix 1: Quality assessment of HLD and HGC primer sets

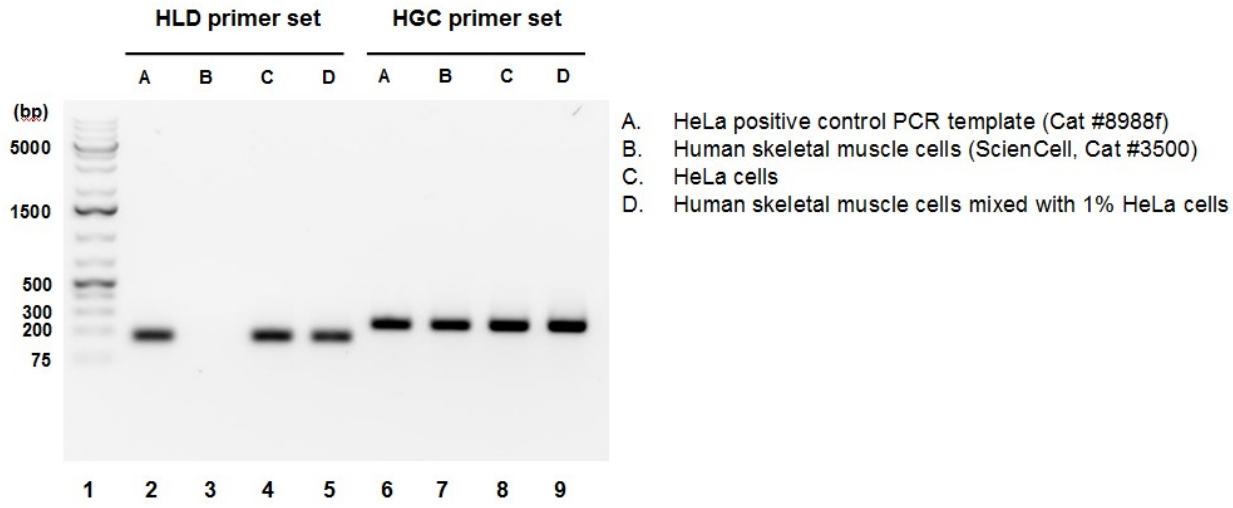

Figure 1. Gel electrophoresis separation of PCR products of 4 samples using this kit on a 1.5% agarose gel. Lane 1: DNA marker; Lanes 2-5: PCR products of genomic DNA samples A-D using HLD primer set. As expected, samples A, C and D yielded a band of 180 bp and sample B did not yield a band; Lanes 6-9: PCR products of genomic DNA samples A-D using HGC primer set. All PCR reactions yielded a band at expected size of 240 bp.

Appendix 2: Results interpretation and troubleshooting

Table 4. Results interpretation
A positive band for HLD primer set is at 180bp. A positive band for HGC primer set is at 240bp.

Observation			**Interpretation**
Sample	**HLD primer set**	**HGC primer set**	
HeLa positive control PCR template (Cat #8988f)	positive	positive	PCR works well
"no-lysate" negative control	negative	negative	No contamination in PCR reagents
target sample	negative	positive	Target sample has no detectable HeLa cell contamination
target sample	positive	positive	Target sample has HeLa genomic DNA contamination

Table 5. Troubleshooting

Observation			**Troubleshooting**
Sample	**HLD primer set**	**HGC primer set**	
HeLa positive control PCR template (Cat #8988f)	negative	negative	PCR failed. Check quality of PCR reagents and PCR program.
"no-lysate" negative control	positive	positive	Reagents were contaminated with HeLa cell genomic DNA. Use new reagents.
"no-lysate" negative control	negative	positive	Reagents were contaminated with non-HeLa cell human genomic DNA. Use new reagents.