SHENTEK

10-Species Cell Line Authentication Kit

User Guide

Version: A/0
For Research Use Only
Product No.: 1801940
Reagents for 50 Reactions

Huzhou Shenke Biotechnology Co., Ltd

(IMPORTANT: Please read this document carefully before experiment.)

1.Product information

■ Product description

Cell identification testing is a mandatory requirement set by the World Health Organization (WHO), the U.S. Food and Drug Administration (FDA), the European Pharmacopoeia, and the Chinese Pharmacopoeia. It is essential for confirming the origin of production cells and ruling out cross-contamination with other cell types. The 10-species Cell Line Authentication Kit provides a rapid, user-friendly and highly effective solution for species verification and contamination detection.

This kit utilizes mitochondrial genes-specifically Cytochrome Oxidase I (CO I), Cytochrome B (Cyt B), or Cytochrome Oxidase II (CO II) as molecular targets for the identification of 10 commonly used cell lines in biologics manufacturing (refer to Table 1). It employs a multiplex PCR assay with species-specific primers that produce distinct amplicon sizes for each cell line (as shown in Figure 1). The results are then analyzed through horizontal DNA electrophoresis, allowing for species identification and potential cross-contamination detection based on the number and size of the amplified bands.

The detection limit for species identification and cross-contamination detection has been validated no more than 1%. The kit enables detect simultaneous detection of 10 species listed in the Table 1 in a single reaction.

Table 1. List of 10 cell species detectable by this kit

Cell species	Common name	Cell species	Common name
Bos taurus	Cow	*Macaca mulatta*	Monkey, rhesus
Mus musculus	Mouse	*Cricetulus griseus*	Hamster, Chinese
Canis familiaris	Dog	*Felis catus*	Cat
Rattus norvegicus	Rat	*Homo sapiens*	Human
Cercopithecus aethiops	Monkey, African green	*Sus scrofa*	Pig

■ Kit contents and storage

WARNING: Please read the Material Safety Data Sheets (MSDSs) and follow the handling instructions. Wear appropriate protective eyewear, mask, clothing, and gloves.

Table 2. Kit components and storage

Reagent	Part No.	Quantity	Storage
DNA Sizing Templates MIX	NNA048	25 μL×2 tubes	-20℃
Cell Extraction Buffer	NNB015	1.25 mL×4 tubes	
2×Multiplex PCR Buffer	NNB016	320 μL×2 tubes	
DNA Sizing Primers MIX	NNC082	120 μL×2 tubes	
DEPC-treated H_2O	NND052	1 mL×2 tubes	
DL500 DNA Marker	/	1 tube	

Note: Store the DL500 DNA Marker at 2-8°C after thawing, aliquot if necessary to avoid repeated freeze-thaw cycles.

The kit components can be stored at appropriate conditions for up to 24 months. Please check the expiration date on the labels.

■ Complementary reagents supplied with the kit

- 6× Loading Buffer (Product No.: 1801942)
- 10× TBE Buffer (Product No.: 1801943)

■ Applied instruments, including but not limited to the following

- BIOER TC-EA Gene Amplifier
- Other PCR instruments that have been validated for suitability (including quantitative PCR or PCR instruments)

■ Required materials not included in the kit

- Low retention, RNase/DNase-free, sterile microcentrifuge tubes, 1.5 mL
- Low retention, RNase/DNase-free, filter tips, 1000μL, 200μL, 100μL and 10μL
- PCR 8-well strip tubes with caps
- Nucleic acid dye
- PBS (Without Mg^{2+} and Ca^{2+}, pH 7.4)
- Agarose

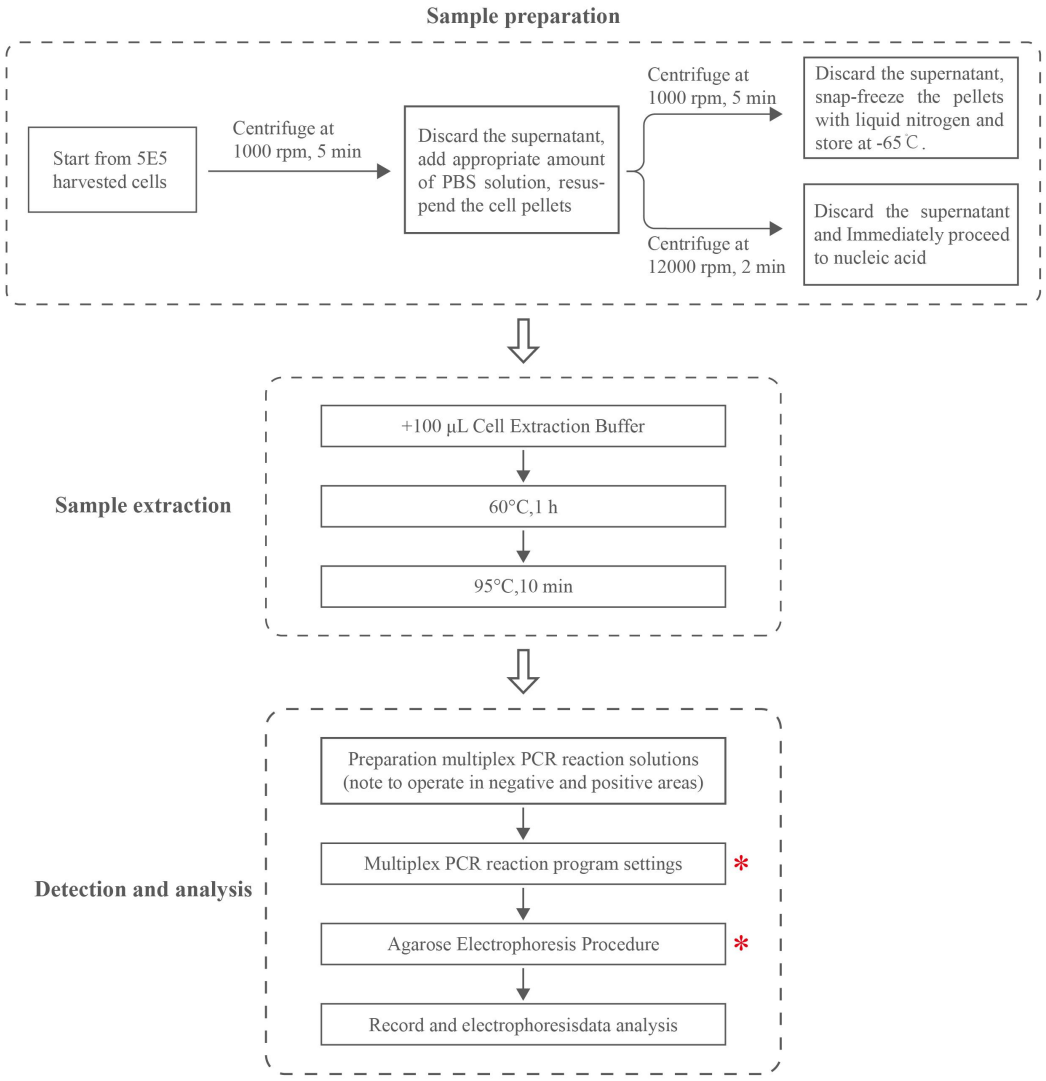
■ Related equipments

- PCR instrument

- Vortex mixer
- Pipettes: 1000 μL, 200μL, 100 μL and 10 μL
- High-speed refrigerated centrifuge and Benchtop microcentrifuge
- Dry bath incubator
- Horizontal electrophoresis tank
- Conical flasks, measuring cylinders and other gel-making tools
- Electrophoresis system power supply
- Gel Imaging Instrument
- Microwave oven

■ Experimental procedure

(Please read this User Guide carefully and completely before experimenting)



Note:1.Pilot Study Recommendation: A pilot study is strongly recommended. This includes verifying the program settings of the PCR instrument and the conditions for horizontal electrophoresis tank. For detailed validation protocols, please contact our technical support team.

2. Laboratory Layout for Contamination Prevention: To avoid cross-contamination, it is advisable to use physically separated rooms for the sample preparation area, positive control area, negative control area, and amplification area. If separate rooms are not available, the sample preparation area, positive control area, and negative control area should be clearly demarcated within a single room. Additionally, the amplification area should be located in a separate room to ensure containment and prevent contamination.

■ Preparation of sample extracts

Note: All operations should be performed in the sample preparation area.

1. Sample Preparation

1.1 Take 5E+05 freshly cultured cells (total number of cells) and transfer them into a 1.5 mL microcentrifuge tube. Centrifuge at 113×g (equivalent to approximately 1000 rpm) for 5 minutes and discard the supernatant completely, then resuspend the cell pellets with approximately 500 μL of PBS (Without Mg^{2+} and Ca^{2+}, pH 7.4).

1.2 The following operations can be performed depending on the actual situation:

① Case 1: If the sample extraction cannot be performed immediately, centrifuge at 113×g for 5 minutes and discard the supernatant completely without disturbing the cell pellets. Freeze the sample in liquid nitrogen for 10-30 seconds, and then store it at -70℃ or below for no more than one year.

② Case 2: If the sample extraction can be performed immediately, centrifuge at 16,260×g (approximately 12,000 rpm) for 2 minutes and discard the supernatant completely without disturbing the cell pellets.

2. Sample Extraction

2.1 Add 100 μL of Cell Extraction Buffer (NNB015) to each test sample, immediately vortex to mix well.

2.2 Incubate in a dry bath at 60℃ for 1 hour, followed by heat-incubation at 95℃ for 10 minutes, then allow to cool to room temperature.

Note: The sample extracts can be stored at -20℃ for up to 1 month, and avoid repeated freeze-thaw cycles.

3. Negative Control Sample (NCS) Preparation

Add 100 μL of Cell Extraction Buffer to a microcentrifuge tube, and follow the same extraction procedure as the test samples.

■ Preparation of multiplex PCR Reaction MIX

1. Preparation of DNA Sizing Templates MIX Working Solution

Note: All operations should be performed in the positive area.

Transfer 18 μL of DEPC-treated H_2O into a new 1.5 mL microcentrifuge tube, and add 2 μL of DNA Sizing Templates MIX (NNA048).

2. Preparation of PCR MIX

Note: All operations should be performed in the negative area.

(1) Calculate the number of reaction wells based on the number of test samples:

Number of reaction wells=1 No Template Control (NTC) + Number of test samples + 1 NCS + 1 DNA Sizing Ladder.

Note: DNA Sizing Ladder is amplified using the DNA Sizing Templates MIX Working Solution as template.

(2) Thaw all reagents on ice or at 2-8°C, and prepare PCR MIX as shown in Table 3:

Table 3. Preparation of PCR MIX

Reagents	Volume/reaction	Volume for 30 reaction (includes 10% overage)
2× Multiplex PCR Buffer	12.5 μL	412.5
DNA Sizing Primers MIX	4.5 μL	148.5
Total Volume	17 μL	561

(3) Mix thoroughly and place on ice, aliquot 17 μL/well into PCR 8-strip tubes.

3. Preparation of PCR Reaction MIX

Prepare qPCR Reaction MIX according to Table 4:

Table 4. Preparation of PCR Reaction MIX

Operation Area	**Negative Area**	**Sample Preparation Area**		**Positive Area**
Tubes	**NTC**	**NCS**	**Test sample**	**DNA Sizing Ladder**
PCR MIX	17 μL	17 μL	17 μL	17 μL
Template	8 μL DEPC-treated H_2O	8 μL Extract NCS	8 μL Extracted sample	8 μL DNA Sizing Templates MIX working solution
Total Volume	25 μL	25 μL	25 μL	25 μL

■ PCR program setting

Set thermal-cycling conditions as in Table 5:

Table 5. PCR reaction program

Step	**Temp.**	**Time**	**Cycles**
1	95℃	5 min	1
2	95℃	30 s	25-30*
3	62℃	3 min	
4	68℃	30 s	
5	68℃	30 min	1
6**	4℃	∞	1

**The number of cycles in steps 2-4 of the reaction program can be adjusted according to the actual situation, 28 cycles are recommended for the pilot study.*

*** For step 6, if the PCR instrument cannot maintained at 4℃, please remove the PCR plate or 8-strip tubes immediately after the program end to prevent degradation of PCR amplification products.*

■ Agarose Electrophoresis Procedure

Agarose electrophoresis should be performed in the designated electrophoresis area.

1. Prepare a 2% agarose gel for the assay as follows:

2% agarose gel (w/v) = agarose (g) / 1×TBE Buffer (mL) × 100% + adequate amount of nucleic acid dye (optional)

Notes: (1) Prepare a 1× TBE Buffer by diluting the 10× TBE Buffer with ultrapure water to a 1× concentration. If the 10× TBE Buffer appears cloudy or contains precipitates, heat it until it becomes clear.

(2) For the amount of nucleic acid dye and the staining method (in-gel or post-electrophoresis), please refer to the product specifications. Choose a staining method that does not alter the migration of the samples.

(3) Specifications for the gel combs and tray: Use well combs with a thickness of 1.5 mm, 25 wells (teeth), and a width of 120 mm. The gel tray should be at least 60 mm in length.

2. Sample Preparation and Electrophoresis Operation

(1) Add an appropriate amount of loading buffer to the PCR amplification products to achieve a final concentration of 1× Loading Buffer. The recommended loading volume is 6-10 μL per well. Include one well with a DL500 DNA marker as a reference.

Notes: Gently open the lid of the PCR tube to avoid cross-contamination from splashing the amplification product. Additionally, please note that the cap should only be opened in the electrophoresis area. The electrophoresis room must be separated from the amplification room and the PCR reaction mix preparation room.

(2) Run the gel at a constant voltage of 80–110 until the bromophenol blue bands migrates near the edge of the gel (approximately 60 mm in length), then stop the run.

Note:To avoid cross-contamination, reseal the opened PCR amplification product with new caps if using 8-strip tubes.

■ Gel imaging

Imaging Parameter Settings

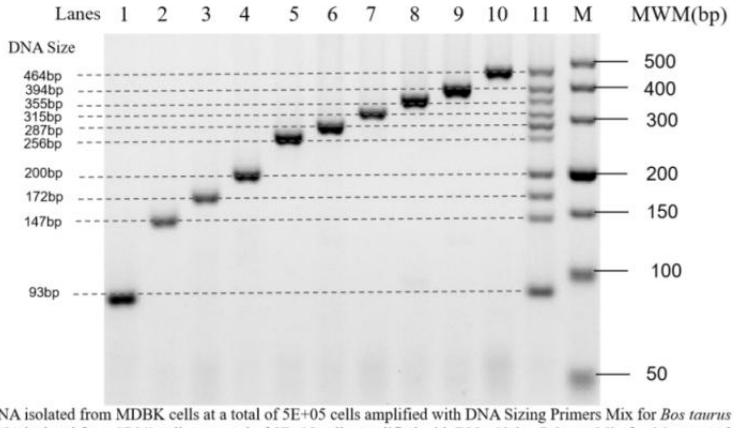
Using the GenoSens2200 Touch gel imaging system (Clinx) as an example for imaging parameters:

• Resolution: Ultra High

• Exposure Intensity: 700 ms

• Display Settings: Zoom in/out to fit, Low 200, High 65535

Note: Imaging parameters may differ among the gel imaging instruments. Make sure the 10 bands of the DNA Sizing Ladder are clear, as shown in lane 11 of Figure 1. If not, please analyze the potential causes and adjust the settings as needed.



1 = DNA isolated from MDBK cells at a total of 5E+05 cells amplified with DNA Sizing Primers Mix for *Bos taurus* (cow) species 93bp
2 = DNA isolated from SP2/0 cells at a total of 5E+05 cells amplified with DNA Sizing Primers Mix for *Mus musculus* (mouse) species 147bp
3 = DNA isolated from MDCK cells at a total of 5E+05 cells amplified with DNA Sizing Primers Mix for *Canis familiaris* (dog) species 172bp
4 = DNA isolated from C6 cells at a total of 5E+05 cells amplified with DNA Sizing Primers Mix for *Rattus norvegicus* (rat) species 200bp
5 = DNA isolated from Vero cells at a total of 5E+05 cells amplified with DNA Sizing Primers Mixfor *Cercopithecus aethiops* (Gr. monkey) species 256bp
6 = DNA isolated from LLC-MK2 cells at a total of 5E+05 cells amplified with DNA Sizing Primers Mix *for Macaca mulatta* (Rhesus monkey) species 287bp
7 = DNA isolated from CHO cells at a total of 5E+05 cells amplified with DNA Sizing Primers Mix for *Cricetulus griseus* (Chinese hamster) species 315bp
8 = DNA isolated from CRFK cells at a total of 5E+05 cells amplified with DNA Sizing Primers Mix for *Felis catus* (cat) species 355bp
9 = DNA isolated from HEK293 cells at a total of 5E+05 cells amplified with DNA Sizing Primers Mix for *Homo sapiens* (human) species 394bp
10 = DNA isolated from PK15 cells at a total of 5E+05 cells amplified with DNA Sizing Primers Mix for *Sus scrofa* (pig) species 464bp
11 = DNA Sizing Templates mix amplified with DNA Sizing Primers Mix for 1-10 cell species DNA Sizing ladder
M =DL500 Marker

Figure 1. Identification of 10 different cell species using the kit

Note: Lanes 1–10 are single-species DNA Ladders amplified from 10 specific cell lines; lane 11 contains PCR products amplified from a mixture of 10 cell lines and also serves as a DNA sizing ladder..

■ Result Analysis

1. No Template Control (NTC) and Negative Control Sample (NCS) should show no detectable amplified bands;
2. DNA Sizing Ladder should be consistent with the size distribution of lane 11 in Fig. 1, with 10 clearly distinguishable bands;
3. If the test sample shows only a single distinct band matching the size of the amplification product from the template cell species (as shown in Fig. 1), the sample is considered free from contamination, and the cell species can be identified.

4. If two or more electrophoretic bands are observed in the test sample lane, and the band size matched those of 10 cell species shown in Table 6, which indicate there are contaminants from other species in the sample, and the identity of the contaminants can be inferred from the size of the amplicons.

Table 6. Band sizes of 10 cell species specific amplicons

Cell species	Common name	Size (bp)
Bos taurus	Cow	93
Mus musculus	Mouse	147
Canis familiaris	Dog	172
Rattus norvegicus	Rat	200
Cercopithecus aethiops	Monkey, African green	256
Macaca mulatta	Monkey, rhesus	287
Cricetulus griseus	Hamster, Chinese	315
Felis catus	Cat	355
Homo sapiens	Human	394
Sus scrofa	Pig	464

5. To ensure the reliability of the assay, it is recommended that each test sample shall be analyzed in two independent replicates.
6. Please contact our technical support if you have any further questions.

Effective date: 05 Jun. 2025

Support & Contact

SHENTEK

Huzhou Shenke Biotechnology Co., Ltd.
www.shentekbio.com
Address: 8th Floor, 6B Building, No.1366 Hongfeng Road, Huzhou 313000, Zhejiang Province, China
E-mail: info@shentekbio.com
Phone: +1 (908) 822-3199 / (+86) 400-878-2189