DirEx™

Single-tube PCR-template DNA preparation solution

Cat. No. 250-101

for research use only

Kit Contents

(100 prep/kit)

Components	Quantity
DirEx™	1.5 ml x 7 tubes
Buffer A	1 ml
Proteinase K	2.2 mg
PK reconstitution buffer	1 ml

Description

GeneAll® DirEx™ is designed for the easy and simple preparation of template DNA in PCR applications. The whole procedure can be completed in a single tube and it takes just 8 minutes. The procedure of DirEx™ is composed of two steps, the incubation and the inactivation, which are the lysis of sample and the heat-inactivation of enzyme respectively. The incubating is normally performed in a conventional water- or dry-bath, but PCR thermal cycler can also be used alternatively. Use of this instrument for incubating can dramatically increase the efficiency and the ease of handling, and it will eventually enhance the consistency and the accuracy of the analysis.

The simple procedure of DirEx™ requires neither the centrifuge step nor the additional pipetting, and it facilitates the multiple preparations from many samples. Simultaneous preparation from many samples with minimum handling will help guarantee the fidelity of the analysis.

GeneAll® DirEx™ can be used for the preparation of template DNA from a wide range of biological and forensic samples, such as mammalian blood, hairs, tissues, swabs, blood stains, cigarette butts and cultured cells.

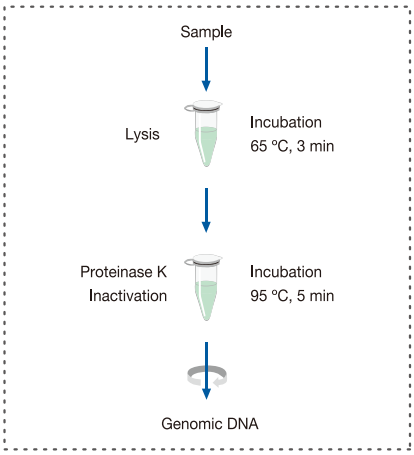
Prepared DNA can be applied directly to PCR applications and / or stored in a freezer for storage.

Proteinase K Reconstitution

This kit provides a lyophilized proteinase K and a PK reconstitution buffer. To obtain a proteinase K solution (20 mg / ml), add 110 ul of the PK reconstitution buffer to the tube containing lyophilized proteinase K and dissolve it thoroughly. After reconstituting, the proteinase K solution can be stored according to the storage condition as below.

Storage conditions

GeneAll® DirEx™ should be stored at room temperature (15°C ~ 25°C). The reconstituted proteinase K can be stored at 4°C for 1 year without significant decrease in activity. But for prolonged preservation of activity, storing under -20°C is recommended.



Required equipments and materials for procedure

- **Using water or dry bath**
  - Prepare sterile 1.5 ml microcentrifuge tubes
  - Set two water or dry bath to 65°C and 95°C
- **Using PCR thermal cycler**
  - Prepare sterile 0.2 ml thin-wall PCR tubes
  - Program PCR thermal cycler as below :
    [65°C, 3 minutes → 95°C, 5 minutes → 4°C, ∞]

Protocol for Animal tissue / Tail snip

1. **Prepare the mixture as below in a 1.5 ml microcentrifuge tube or 0.2 ml PCR tube.**
   - ▸ 100 ul of DirEx™
   - ▸ 1 ul of Proteinase K (20 mg / ml)
   - ▸ 10 ul of Buffer A
2. **Place 10 mg of animal tissues or tail snips in the mixture.**
   Especially, tail snip should be minced finely with sterile scissors or scalpel.
3. **Vortex to mix for 15 seconds.**
   If the sample is attached on lid or wall surface of the tube after vortex, spin down briefly to collect the samples to bottom of the tube.
4. **Incubate at 65°C for 3 minutes and then 95°C for 5 minutes.**
   Either a PCR thermal cycler or water/dry bath can be used for this step.
5. **Vortex to mix for 10 seconds and spin down briefly to remove any drops from inside of the lid.**
6. **Use the supernatant immediately as template DNA for analysis, or transfer the supernatant to a new tube for storage.**
   For best results in PCR, it is recommended to use 1~2 ul of the prepared DNA solution for 20 ul PCR reaction.

Protocol for Whole blood / Dried blood spot

1. Prepare the mixture as below in a 1.5 ml microcentrifuge tube or 0.2 ml PCR tube.

For mammalian whole blood	For dried blood spot
100 ul of DirEx™ 1 ul of Proteinase K (20 mg / ml) 10 ul of Buffer A	100 ul of DirEx™

2. Place blood sample in the mixture.
   In order to ensure efficient PCR, it is essential that optimized volume of sample is applied as follow :
   - Mammalian whole blood : 20 ul
   - Dried blood spot : A 5 mm punch-out disc from dried blood spot

3. Vortex to mix for 15 seconds.
   If the sample is attached on lid or wall surface of the tube after vortex, spin down briefly to collect the samples to bottom of the tube.

4. Incubate at 65ºC for 3 minutes and then 95ºC for 5 minutes.
   Either a PCR thermal cycler or water/dry bath can be used for this step.

5. Vortex to mix for 10 seconds. Spin down briefly to remove any drops from inside of the lid (Dried blood spot) or centrifuge at 13,000 rpm for 30 seconds to eliminate cell debris (Mammalian whole blood).
   The debris containing PCR inhibitors should be removed from the lysate. Centrifugation at 13,000 rpm for 30 seconds is sufficient to pellet unnecessary debris of blood.

6. Use the supernatant immediately as template DNA for analysis, or transfer the supernatant to a new tube for storage.
   For best results in PCR, it is recommended to use 1~2 ul of the prepared DNA solution for 20 ul PCR reaction.

Protocol for Cigarette butt

1. Prepare the mixture as below in a 1.5 ml microcentrifuge tube or 0.2 ml PCR tube.
   - 100 ul of DirEx™
   - 1 ul of Proteinase K (20 mg / ml)

2. Cut off a 1x1 cm piece of outer filter paper from the end of cigarette butts and submerge the piece in the mixture.

3. Vortex to mix for 15 seconds.
   If the sample is attached on lid or wall surface of the tube after vortex, spin down briefly to collect the samples to bottom of the tube.

4. Incubate at 65ºC for 3 minutes and then 95ºC for 5 minutes
   Either a PCR thermal cycler or water/dry bath can be used for this step.

5. Vortex to mix for 10 seconds and spin down briefly to remove any drops from inside of the lid.

6. Use the supernatant immediately as template DNA for analysis, or transfer the supernatant to a new tube for storage.
   For best results in PCR, it is recommended to use 1~2 ul of the prepared DNA solution for 20 ul PCR reaction.

Protocol for Cultured cell

1. Prepare the mixture as below in a 1.5 ml microcentrifuge tube or 0.2 ml PCR tube.
   - 100 ul of DirEx™
   - 1 ul of proteinase K (20 mg / ml)

2. Place cultured cells in the mixture.
   In order to ensure efficient PCR, it is essential that optimized volume of sample is applied as follow :
   - Mammalian cells : 10 ul of cell suspension containing up to $5x10^6$ cells
   - Bacterial cells : 15 ul of cell suspension (OD_{600nm} =1.5) or one single colony picked from a solid media

3. Vortex to mix for 15 seconds.
   If the sample is attached on lid or wall surface of the tube after vortex, spin down briefly to collect the samples to bottom of the tube.

4. Incubate at 65ºC for 3 minutes and then 95ºC for 5 minutes.
   Either a PCR thermal cycler or water/dry bath can be used for this step.

5. Vortex to mix for 10 seconds and spin down briefly to remove any drops from inside of the lid.

6. Use the supernatant immediately as template DNA for analysis, or transfer the supernatant to a new tube for storage.
   For best results in PCR, it is recommended to use 1~2 ul of the prepared DNA solution for 20 ul PCR reaction.

Protocol for Buccal swab / Hair

1. Prepare the mixture as below in a 1.5 ml microcentrifuge tube or 0.2 ml PCR tube.
   - 100 ul of DirEx™
   - 1 ul of Proteinase K (20 mg / ml)

2. Place buccal swab or hair follicle/shaft in the mixture.
   In order to ensure efficient PCR, it is essential that optimized amount of sample is applied as follow :
   - Buccal swab : A head of cotton swab scraped more than 5-6 times against the inside of cheek.
   - Hair follicle / shaft : 2~3 hair roots or shafts of 1 cm in length from plucked hair

3. Vortex to mix for 15 seconds.
   If the sample is attached on lid or wall surface of the tube after vortex, spin down briefly to collect the samples to bottom of the tube.

4. Incubate at 65ºC for 3 minutes and then 95ºC for 5 minutes.
   Either a PCR thermal cycler or water/dry bath can be used for this step.

5. Vortex to mix for 10 seconds and spin down briefly to remove any drops from inside of the lid.

6. Use the supernatant immediately as template DNA for analysis, or transfer the supernatant to a new tube for storage.
   For best results in PCR, it is recommended to use 1~2 ul of the prepared DNA solution for 20 ul PCR reaction.