Genomic DNA Isolation Reagent Kit
(Blood/Cultured Cell/Tissue)

Cat No. PDR05-0100(S)
Size: 100 Reactions

Sample: 300 µl of the whole blood 200 µl of the buffy coat
Up to 10^7 cells of the mammalian cells
Up to 10^9 cells of the bacterial cells Up to 10^8 cells of the fungus cells
30 mg of the animal tissue
Operation time: within 60 minutes

Description

The Genomic DNA Isolation Reagent Kit (Blood/Cultured Cell/Tissue) is a reagent system kit. The kit is designed specifically for genomic DNA isolation from the whole blood, frozen blood, buffy coat, cultured animal/bacterial cells, fungus cells and tissue. This unique reagent system ensures genomic DNA with high yield and good quality from samples. The entire procedure can be completed in 1 hour without phenol/ chloroform extraction. Purified genomic DNA is suitable for use in PCR or other enzymatic reactions.

Kit Contents

Contents	PDR05-0100	PDR05-0100S
Buffer BR	100 ml	4 ml
Buffer BC	35 ml	1.5 ml
Buffer BP	12 ml	500 µl

Feature

- Delivering high-quality genomic DNA with the fast procedure
- Ready-to-use genomic DNA for high performance in any downstream application
- Highly purified and high yield genomic DNA can be extracted from various samples
- Optimized lysis buffer for the efficient lysis
- Designed to rapidly purify high-quality DNA using spin reagent format

Application

- Gene cloning
- Southern blotting
- PCR
- SNP genotyping

Quality Control

The quality of the Genomic DNA Isolation Reagent Kit (Blood/Cultured Cell/Tissue) is tested on a lot-to-lot basis to ensure consistent product quality.

Required Materials

- Microcentrifuge tubes
- RNase A (10 mg/ml)
- For the tissue sample: Proteinase K (10 mg/ml), Micropestle
- Isopropanol
- TE Buffer
- For the Gram-positive bacteria sample: lysozyme buffer (20 mg/ml lysozyme; 20 mM Tris-HCl; 2 mM EDTA; 1% TritonX-100; pH 8.0, prepare the lysozyme buffer immediately prior to use)
- For the fungus sample: lyticase or zymolase, sorbitol buffer (1.2 M sorbitol;10 mM $CaCl_2$; 0.1 M Tris-HCl pH 7.5, 35 mM β- mercaptoethanol)

Protocol

Fresh whole Blood or Buffy Coat

Step 1 Sample Cells Harvesting

1. Collect blood in EDTA-Na_2 treated collection tubes (or other anticoagulant mixtures).
2. Transfer up to 300 µl of the blood or 200 µl of the buffy coat to a sterile 1.5 ml microcentrifuge tube.
3. Add 900 µl of the Buffer BR and mix by inversion.
4. Incubate the tube at the room temperature for 10 minutes (invert twice during incubation).
5. Centrifuge for 5 minutes at 4,000 xg.
6. Remove the supernatant completely and resuspend the cells in 50 µl of the Buffer BR by pipetting the pellet.

Step 2 Lysis

1. Add 300 µl of the Buffer BC to the resuspended cells from Step 1 and mix by vortex.
2. Incubate at 60°C for 10 minutes or until the sample lysate is clear.
3. During incubation, invert the tube every 3 minutes.

Optional Step:

4. RNA Degradation (If RNA-free genomic DNA is required, perform this optional step): Add 5 µl of the RNase A (10 mg/ml) to the sample lysate and mix by vortex. Incubate at the room temperature for 5 minutes.

Step 3 Protein Removal

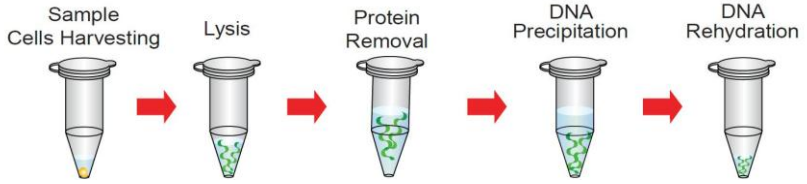
1. Add 100 µl of the Buffer BP to the sample lysate and vortex immediately for 10 seconds.
2. Incubate on ice for 5 minutes.
3. Centrifuge at 14-16,000 xg for 3 minutes.
4. Transfer the supernatant from Step 3 to a clean 1.5 ml microcentrifuge tube.

Step 4 DNA Precipitation

1. Add 300 µl of the Isopropanol to the sample from Step 3 and mix well by inverting 20 times.
2. Centrifuge at 14-16,000 xg for 5 minutes.
3. Discard the supernatant carefully and add 300 µl of the 70% ethanol to wash the pellet.
4. Centrifuge at 14-16,000 xg for 3 minutes.
5. Discard the supernatant carefully and air-dry the pellet for 5 minutes.

Step 5 DNA Rehydration

1. Add 50-100 µl of the TE buffer or distilled water and incubate at 60°C for 5-10 minutes to dissolve the DNA pellet. During incubation, tap the bottom of the tube to promote DNA rehydration.



Protocol

Cultured Mammalian Cells

Step 1 Sample Cells Harvesting
1. Transfer cultured mammalian cells (up to 10^7) to a sterile 1.5 ml microcentrifuge tube.
2. Centrifuge at 6,000 xg for 1 minute.
3. Remove the supernatant completely and resuspend the cells in 50 µl of the Buffer BR by pipetting the pellet.

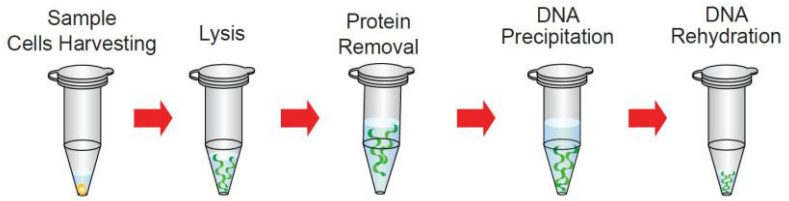
Step 2 Lysis
1. Add 300 µl of the Buffer BC to the resuspended cells from Step 1 and mix by vortex.
2. Incubate at 60°C for 10 minutes or until the sample lysate is clear.
3. During incubation, invert the tube every 3 minutes.

Optional Step:
4. RNA Degradation (If RNA-free genomic DNA is required, perform this optional step): Add 5 µl of the RNase A (10 mg/ml) to the sample lysate and mix by vortex. Incubate at the room temperature for 5 minutes.

Step 3 Protein Removal
1. Add 100 µl of the Buffer BP to the sample lysate and vortex immediately for 10 seconds.
2. Incubate on ice for 5 minutes.
3. Centrifuge at 14-16,000 xg for 3 minutes.
4. Transfer the supernatant from Step 3 to a clean 1.5 ml microcentrifuge tube.

Step 4 DNA Precipitation
1. Add 300 µl of the Isopropanol to the sample from step 3 and mix well by inverting 20 times.
2. Centrifuge at 14-16,000 xg for 5 minutes.
3. Discard the supernatant carefully and add 300 µl of the 70% ethanol to wash the pellet.
4. Centrifuge at 14-16,000 xg for 3 minutes.
5. Discard the supernatant carefully and air-dry the pellet for 5 minutes.

Step 5 DNA Rehydration
1. Add 50-100 µl of the TE buffer or distilled water and incubate at 60°C for 5~10 minutes to dissolve the DNA pellet. During incubation, tap the bottom of the tube to rehydration.



Gram-Negative Bacterial Cells

Step 1 Sample Cells Harvesting
1. Transfer cultured bacterial cells (up to 10^9) to a sterile 1.5 ml microcentrifuge tube.
2. Centrifuge at 12,000 xg for 1 minute.
3. Remove the supernatant completely and resuspend the cells in 50 µl of the Buffer BR by pipetting the pellet.

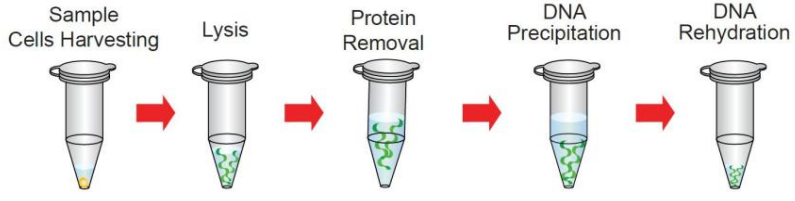
Step 2 Lysis
1. Add 300 µl of Buffer BC to the resuspended cells from Step 1 and mix by vortex.
2. Incubate at 60°C for 10 minutes or until the sample lysate is clear.
3. During incubation, invert the tube every 3 minutes.

Optional Step:
4. RNA Degradation (If RNA-free genomic DNA is required, perform this optional step): Add 5 µl of the RNase A (10 mg/ml) to the sample lysate and mix by vortex. Incubate at room temperature for 5 minutes.

Step 3 Protein Removal
1. Add 100 µl of the Buffer BP to the sample lysate and vortex immediately for 10 seconds.
2. Incubate on ice for 5 minutes.
3. Centrifuge at 14-16,000 xg for 3 minutes.
4. Transfer the supernatant from Step 3 to a clean 1.5 ml microcentrifuge tube.

Step 4 DNA Precipitation
1. Add 300 µl of the Isopropanol to the sample from Step 3 and mix well by inverting 20 times.
2. Centrifuge at 14-16,000 xg for 5 minutes.
3. Discard the supernatant carefully and add 300 µl of the 70% ethanol to wash the pellet.
4. Centrifuge at 14-16,000 xg for 3 minutes.
5. Discard the supernatant carefully and air-dry the pellet for 5 minutes.

Step 5 DNA Rehydration
1. Add 50-100 µl of the TE buffer or distilled water and incubate at 60°C for 5~10 minutes to dissolve the DNA pellet. During incubation, tap the bottom of the tube to promote DNA rehydration.



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Protocol

Gram-Postive Bacterial Cells

Step 1 Sample Cells Harvesting

1. Transfer cultured bacterial cells (up to 10^9) to a sterile 1.5 ml microcentrifuge tube.
2. Centrifuge at 12,000 x g for 1 minute.
3. Remove the supernatant completely and resuspend the cells in 100 µl of the lysozyme buffer by pipetting the pellet. Incubate at the room temperature for 20 minutes.

Step 2 Lysis

1. Add 300 µl of the Buffer BC to the resuspended cells from Step 1 and mix by vortex.
2. Incubate at 60°C for 10 minutes or until the sample lysate is clear.
3. During incubation, invert the tube every 3 minutes.

Optional Step:

4. RNA Degradation (If RNA-free genomic DNA is required, perform this optional step): Add 5 µl of the RNase A (10 mg/ml) to the sample lysate and mix by vortex. Incubate at room temperature for 5 minutes.

Step 3 Protein Removal

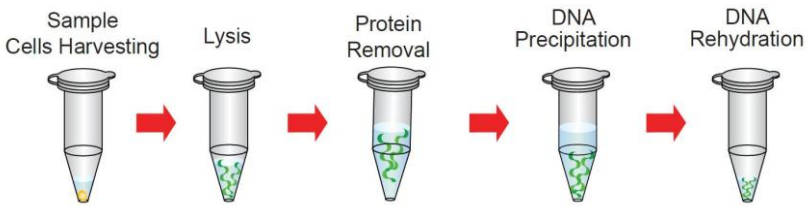
1. Add 100 µl of the Buffer BP to the sample lysate and vortex immediately for 10 seconds.
2. Incubate on ice for 5 minutes.
3. Centrifuge at 14-16,000 x g for 3 minutes.
4. Transfer the supernatant from Step 3 to a clean 1.5 ml microcentrifuge tube.

Step 4 DNA Precipitation

1. Add 300 µl of the Isopropanol to the sample from step 3 and mix well by inverting 20 times.
2. Centrifuge at 14-16,000 x g for 5 minutes.
3. Discard the supernatant carefully and add 300 µl of the 70% ethanol to wash the pellet.
4. Centrifuge at 14-16,000 x g for 3 minutes.
5. Discard the supernatant carefully and air-dry the pellet for 5 minutes.

Step 5 DNA Rehydration

1. Add 50-100 µl of the TE buffer or distilled water and incubate at 60°C for 5~10 minutes to dissolve the DNA pellet. During incubation, tap the bottom of the tube to rehydration.



Fungus Cells

Step 1 Sample Cells Harvesting

1. Transfer fungus cells (up to 10^8) to a sterile 1.5 ml microcentrifuge tube.
2. Centrifuge at 6,000 x g for 5 minutes.
3. Remove the supernatant completely and resuspend the cells in 600 µl of the sorbitol buffer by pipetting the pellet.
4. Add 200 U of the lyticase or zymolase.
5. Incubate at 30°C for 30 minutes.
6. Centrifuge the mixture for 10 minutes at 2,000 xg to harvest the spheroplast.
7. Remove the supernatant completely and resuspend the cells in 50 µl of the Buffer BR by pipetting the pellet.

Step 2 Lysis

1. Add 300 µl of the Buffer BC to the resuspended cells from Step 1 and mix by vortex.
2. Incubate at 60°C for 10 minutes or until the sample lysate is clear.
3. During incubation, invert the tube every 3 minutes.

Optional Step:

4. RNA Degradation (If RNA-free genomic DNA is required, perform this optional step): Add 5 µl of the RNase A (10 mg/ml) to the sample lysate and mix by vortex. Incubate at the room temperature for 5 minutes.

Step 3 Protein Removal

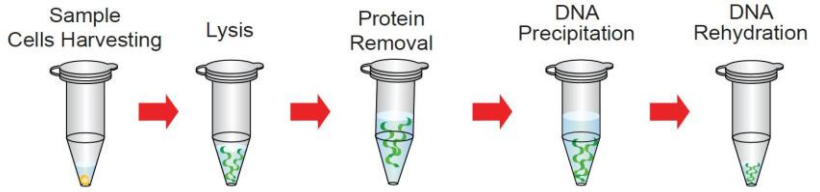
1. Add 100 µl of the Buffer BP to the sample lysate and vortex immediately for 10 seconds.
2. Incubate on ice for 5 minutes.
3. Centrifuge at 14-16,000 xg for 3 minutes.
4. Transfer the supernatant from Step 3 to a clean 1.5 ml microcentrifuge tube.

Step 4 DNA Precipitation

1. Add 300 µl of the Isopropanol to the sample from Step 3 and mix well by inverting 20 times.
2. Centrifuge at 14-16,000 xg for 5 minutes.
3. Discard the supernatant carefully and add 300 µl of the 70% ethanol to wash the pellet.
4. Centrifuge at 14-16,000 xg for 3 minutes.
5. Discard the supernatant carefully and air-dry the pellet for 5 minutes.

Step 5 DNA Rehydration

1. Add 50-100 µl of the TE buffer or distilled water and incubate at 60°C for 5~10 minutes to dissolve the DNA pellet. During incubation, tap the bottom of the tube to rehydration.



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Animal Tissue

Step 1 Sample Tissue Harvesting

1. Transfer 30 mg animal tissue to a sterile 1.5 ml microcentrifuge tube.
2. Use a micropestle to grind the tissue a few times.

Step 2 Lysis

1. Add 300 µl of Buffer BC and 20 µl of the Proteinase K (10 mg/ml) to the tube from Step 1 and continually homogenize the sample tissue with grinding.
2. Incubate at 70°C for 20~30 minutes or until the sample lysate is clear.
3. During incubation, invert the tube every 5 minutes.

Optional Step:

4. RNA Degradation (If RNA-free genomic DNA is required, perform this optional step): Add 5 µl of the RNase A (10 mg/ml) to the sample lysate and mix by vortex. Incubate at the room temperature for 5 minutes.

Step 3 Protein Removal

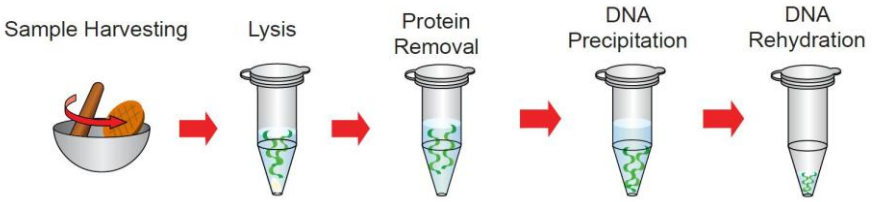
1. Add 100 µl of the Buffer BP to the sample lysate and vortex immediately for 10 seconds.
2. Incubate on ice for 5 minutes.
3. Centrifuge at 14-16,000 xg for 3 minutes.
4. Transfer the supernatant from Step 3 to a clean 1.5 ml microcentrifuge tube.

Step 4 DNA Precipitation

1. Add 300 µl of the Isopropanol to the sample from Step 3 and mix well by inverting 20 times.
2. Centrifuge at 14-16,000 xg for 5 minutes.
3. Discard the supernatant carefully and add 300 µl of the 70% ethanol to wash the pellet.
4. Centrifuge at 14-16,000 xg for 3 minutes.
5. Discard the supernatant carefully and air-dry the pellet for 5 minutes.

Step 5 DNA Rehydration

1. Add 50-100 µl of the TE buffer or distilled water and incubate at 60ºC for 5~10 minutes to dissolve the DNA pellet. During incubation, tap the bottom of the tube to promote DNA rehydration.



Troubleshooting

Refer to the table below to troubleshoot problems that you may encounter when purifying the genomic DNA with the kit.

Problem	Cause	Solution
Low yield of DNA	Incomplete lysed sample	Use the appropriate method for the lysate preparation based on the amount of the starting materials.
		Be sure to add Proteinase K during lysis.
		Increase the digestion time or amount of Proteinase K used for lysis.
		For tissues, cut the tissue into smaller pieces and ensure the tissue is completely immersed in the Lysis step to obtain optimal lysis.
DNA degrade	Sample not fresh	Avoid repeated freeze / thaw cycles of the sample.
		Use a new sample for the DNA isolation. Perform the extraction of the fresh material when possible. The yield and quality of DNA isolated is dependent on the type and age of the starting material.
	Inappropriate sample storage conditions	Store mammalian tissues at -80°C and bacteria at -20°C until use. The whole blood can be stored at 4°C for no longer than 1-2 days.
	DNase contaminant	Use the fresh TAE or TBE electrophoresis buffer.
		Maintain a sterile work environment to avoid contamination from DNases.
Presence of RNA	RNA contamination	Perform RNase A digestion step during the Step Lysis.
Inhibition of downstream enzymatic reactions	Presence of ethanol in purified DNA	Before the DNA Rehydration step, ensure the ethanol was removed completely.

Caution:

1. During operation, always wear a lab coat, disposable gloves, and protective equipment.
2. Check buffers before use for salt precipitation. Redissolve any precipitate by warming to 37°C.
3. Research Use Only. Not intended for any animal or human therapeutic or diagnostic uses.