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

Cat. No.: SN022-0100 Size: 100 Reactions
Cat. No.: SN022-0004 Size: 4 Reactions
Sample: 300 µl of the whole blood
200 µl of the buffy coat
Mammalian cells (up to 1 x $10^7$)
Bacterial cells (up to 1 x $10^9$)
Fungus cells (up to 1 x $10^8$)
30 mg of animal tissues
Format: Reagent
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 one hour without phenol/ chloroform extraction. Purified genomic DNA is suitable for use in PCR or other enzymatic reactions.

## Features
- Fast, reproducible and easy processing by using reagent or spin column system.
- To isolate high quality genomic DNA.
- Isolated genomic DNA is compatible with various downstream applications.

## Applications
- Restriction enzyme digestion.
- Southern blotting.
- PCR amplification.
- Real-Time PCR assay.

## Kit Contents

| Contents | SN022-0100 | SN022-0004 |
|---|---|---|
| Buffer BR | 100 ml X 1 bottle | 2 ml X 2 vials |
| Buffer BC | 35 ml X 1 bottle | 1.5 ml X 1 vial |
| Buffer BP | 12 ml X 1 bottle | 0.5 ml X 2 vials |

## 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
- RNase A (10 mg/ml)
- 1.5 ml Microcentrifuge tubes
- Water bath/ Dry bath
- Air-dry equipments
- Absolute ethanol
- Isopropanol
- For the tissue sample: Proteinase K (10 mg/ml), Micropestle

## Buffer Preparation
- TE buffer, pH8.0 (Selective): 10 mM Tris-HCl, pH 8.0 with 1 mM EDTA
- 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 ß - mercaptoethanol)

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

**Fresh whole Blood or Buffy Coat**

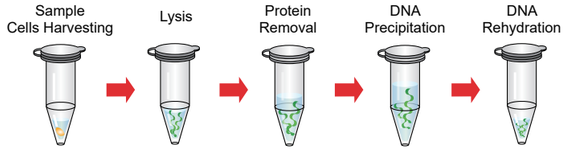
**Step 1 Sample Cells Harvesting**
1. Collect blood in EDTA-$Na_2$ treated collection tubes (or other anticoagulant mixtures).
2. Transfer up to 300 µl of the blood or 200 µl of the buffy coat to a sterile 1.5 ml microcentrifuge tube.
3. Add 900 µl of the Buffer BR and mix by inversion.
4. ncubate the tube at the room temperature for 10 minutes (invert twice during incubation).
5. Centrifuge for 5 minutes at 4,000 x g.
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.
4. Optional Step:
   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 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 (not provided) 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.




**Cultured Mammalian Cells**

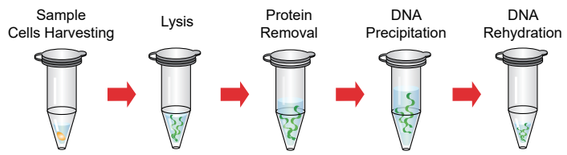
**Step 1 Sample Cells Harvesting**
1. Transfer cultured mammalian cells (up to $10^7$) to a sterile 1.5 ml microcentrifuge tube.
2. Centrifuge at 6,000 x g for 1 minute.
3. Remove the supernatant completely and resuspend the cells in 50 µl of the Buffer BR by pipetting the pellet.

**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.
4. Optional Step:
   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 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 (not provided) 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**

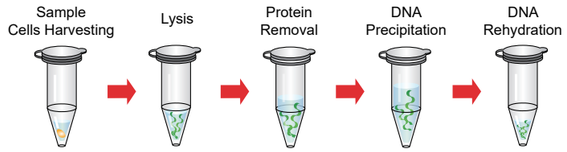
**Step 1 Sample Cells Harvesting**
1. Transfer cultured bacterial cells (up to $10^9$) to a sterile 1.5 ml microcentrifuge tube.
2. Centrifuge at 12,000 x g for 1 minute.
3. Remove the supernatant completely and resuspend the cells in 50 µl of the Buffer BR by pipetting the pellet.

**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.
4. Optional Step:
   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 (not provided) 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.




**Gram-Postive Bacterial Cells**

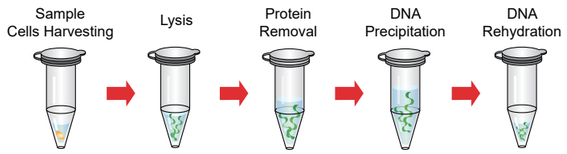
**Step 1 Sample Cells Harvesting**
1. Transfer cultured bacterial cells (up to $10^9$) to a sterile 1.5 ml microcentrifuge tube.
2. Centrifuge at 12,000 x g for 1 minute.
3. Remove the supernatant completely and resuspend the cells in 100 µl of the lysozyme buffer by pipetting the pellet. Incubate at 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.
4. Optional Step:
   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 (not provided) 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.




**<u>Fungus Cells</u>**

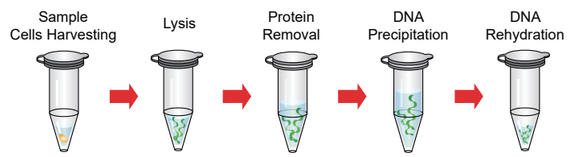
**Step 1 Sample Cells Harvesting**
1. Transfer fungus cells (up to $10^8$) to a sterile 1.5 ml microcentrifuge tube.
2. Centrifuge at 6,000 x g for 5 minute.
3. Remove the supernatant completely and resuspend the cells in 600 µl of the sorbitol buffer by pipetting the pellet.
4. Add 200 U of the lyticase or zymolase.
5. Incubate at 30°C for 30 minutes.
6. Centrifuge the mixture for 10 minutes at 2,000 x g 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.
4. Optional Step:
   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 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 (not provided) 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.




**<u>Animal Tissue</u>**

**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.
4. Optional Step:
   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 x g for 3 minutes.
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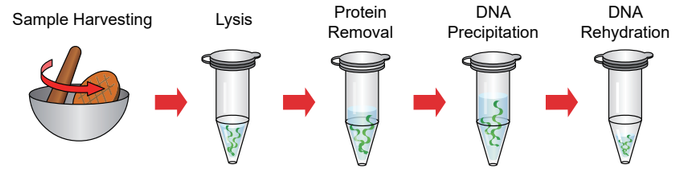
4. Centrifuge at 14-16,000 x g for 3 minutes.
5. Discard the supernatant carefully and air-dry the pellet for 5 minutes.

**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 promote DNA rehydration.




## Troubleshooting

Refer to the table below to troubleshoot problems that you may encounter when you did genomic DNA isolation 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. |
| | | For the tissue samples, add Proteinase K during lysis. |
| | | For the tissue samples, cut the tissue into smaller pieces and ensure the tissue is completely immersed in the Lysis step to obtain optimal lysis. |
| DNA degrade | Sample is 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 condition | 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 DNase. |
| 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. |

## Related Ordering Information

| Cat. No. | Description | Size |
|---|---|---|
| SM101-0500 | *Taq* DNA polymerase | 500 U |
| SM200-0100 | PCR SUPERMIX | 100 Reactions |
| SM201-0100 | Hot Start SUPERMIX | 100 Reactions |
| SM255-0100 | Ultrapure Proteinase K | 100 mg |

## Caution

- During the operation, always wear the latex or vinyl gloves while handling reagents and samples to prevent the DNase contamination.
- Check Buffers before use for salt precipitation. Re-dissolve any precipitate by warming up to 37°C.
- All products are for research use only.