A. PROTOCOL FOR BLOOD AND BODY FLUID /CULTURED CELLS USING MICROCENTRIFUGE

Before experiment

- *Before using for the first time, add absolute ethanol (ACS grade or better) into buffer BW and TW as indicated on the bottle.*
- *Prepare the water bath to 56°C.*
- *Prepare absolute ethanol.*
- *Prepare 1.5 ml microcentrifuge tube.*
- *Equilibrate buffer AE to room temperature.*
- *All centrifugation should be performed at room temperature.*
- *If a precipitate has formed in buffer BL, heat to dissolve at 56°C before use.*

1. Pipet 20 ul of Proteinase K solution (20 mg/ml, provided) into the bottom of a 1.5 ml microcentrifuge tube.

If the sample volume is larger than 200 ul, increase the amount of Proteinase K proportionally.

When the concentration of cells is low, up to 400 ul of starting sample can be used. For 400 ul of sample volume, 40 ul of Proteinase K solution is needed.

2. Transfer 200 ul of sample to the tube. Use the starting sample listed below.

If the sample volume is less than 200 ul, adjust the volume to 200 ul with 1X PBS.

Sample	Max. amount per prep	Preparation
Mammalian whole blood	200 ul	Direct use
Body fluid	200 ul	Direct use
Buffy coat	200 ul	Direct use
Nucleated blood of bird, fish, reptile and amphibian	10 ul	10 ul blood with 190 ul of 1X PBS
Cultured cells or lymphocyte	5 x 10^6 cells	5 x 10^6 cells in 200 ul of 1X PBS
Virus	200 ul	200 ul of virus-containing media

This protocol can be used with Blood/Clinic/Cell SV mini

3. *(Optional :)* **If RNA-free DNA is required, add 20 ul of RNase A solution (20 mg/ml, Cat. No. 391-001, not provided) to the sample, pipet 2~3 times to mix and incubate for 2 min at room temperature.**
Unless RNase A is treated, RNA will be copurified with DNA. RNA can inhibit some downstream enzymatic reactions, but will not inhibit PCR itself.

4. **Add 200 ul of buffer BL to the tube. Vortex the tube to mix thoroughly. Incubate at 56°C for 10 min. Spin down briefly to remove any drops from inside of the lid.**
If the sample volume is larger than 200 ul, increase the volume of buffer BL in proportion. Ratio of buffer BL to the starting sample volume is 1:1.
It is essential to mix the sample and buffer BL thoroughly for good result.
Longer incubation will not affect DNA recovery.

5. **Add 200 ul of absolute ethanol (not provided) to the sample, Pulse-vortex to mix the sample thoroughly, and spin down briefly to remove any drops from inside of the lid.**
If the sample volume is larger than 200 ul, increase the ethanol volume proportionally.

6. **Transfer the mixture to the SV column carefully, centrifuge for 1 min at 6,000 x g above (>8,000 rpm), and replace the collection tube with new one (provided).**
If starting sample volume is larger than 200 ul, apply the mixture twice; apply 700 ul of the mixture, spin down, discard the pass-through, re-insert empty collection tube, and repeat this step again until all of the mixture has applied to the SV column.
If the mixture has not passed completely through the membrane, centrifuge again at full speed (>13,000 x g) until all of the solution has passed through. Centrifugation at full speed is recommended to avoid clogging especially when applying the sample with high-cell density, such as buffy coat, lymphocyte or cultured cells. Centrifugation at full speed will not affect DNA recovery.

7. **Add 600 ul of buffer BW, centrifuge for 1 min at 6,000 x g above (>8,000 rpm) and replace the collection tube with new one (provided).**
If the SV column has colored residue after centrifuge, repeat this step until no colored residue remain. See Troubleshooting guide for detail.
Centrifugation at full speed will not affect DNA recovery.

8. **Apply 700 ul of buffer TW. Centrifuge for 1 min at 6,000 x g above (>8,000 rpm). Discard the pass-through and reinsert the SV column back into the collection tube.**
Centrifugation at full speed will not affect DNA recovery.

9. **Centrifuge at full speed for 1 min to remove residual wash buffer. Place the SV column in a fresh 1.5 ml microcentrifuge tube (not provided).**
Care must be taken at this step for eliminating the carryover of buffer TW.
If a carryover of buffer TW still occurs, centrifuge again for 1 min at full speed with the collection tube before transferring to a new 1.5 ml microcentrifuge tube.
Centrifugation must be performed at full speed (13,000~20,000 x g).

10. Add 200 ul of buffer AE or sterilized water. Incubate for 1 min at room temperature. Centrifuge at full speed for 1 min.

** For low cell-density sample, such as body fluids or virus, use 50~150 ul elution buffer as based on the species and conditions of starting sample or the down-stream applications.*

Ensure that the buffer AE or sterilized water is dispensed directly onto the center of SV column membrane for optimal elution of DNA.

Repeat of elution step with fresh 200 ul elution buffer will increase the total DNA yield significantly, while a third elution step with a further 200 ul of elution buffer will increase yields slightly. Each eluate can be separated in fresh tubes or can be collected to same tube, but more than 300 ul of eluate can not be collected in a 1.5 ml microcentrifuge tube because the SV column will come into contact with the eluate.

If higher concentration of DNA is needed or starting sample amount is very small, second elution can be carried out with first eluate instead of fresh elution buffer. Alternatively for higher concentration, elution volume can be decreased to 50 ul. However the small volume of elution buffer will decrease the total yield of DNA recovery.

For long-term storage, eluting in buffer AE is recommended. But EDTA included in buffer AE can inhibit subsequent enzymatic reactions, so you can avoid such latent problem by using distilled deionized water (>pH 7.0) or Tris-HCl (>pH 8.5). *When using water for elution, check the pH of water before elution.*