Quick Protocol

PCR-Pure Kit

PCR Purification based on silica technology

- MiniPrep

Kit Content

Component	50 preps	100 preps
RPB	28ml	2 x 28ml
GWB	10ml	2 x 10ml
RSB	5ml	10ml
HiPure DR column	50	100
Collection tube	50	100

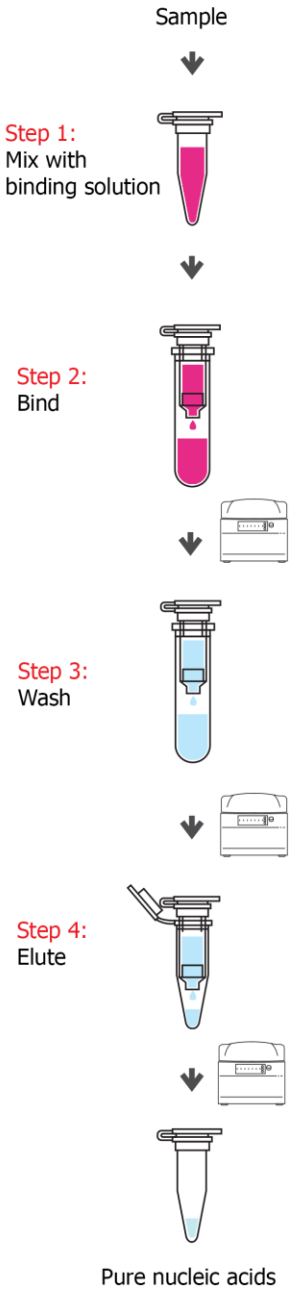
Buffer Preparation

Before the first use, add appropriate amount of ethanol (96-100%) to each washing buffer tube and RPB tube, then mix thoroughly to prepare buffers, refer to Table 1. Do not forget to tick the check box on the bottle label to indicate that ethanol has been added. Before each use mix reconstituted buffer by shaking. Storing at room temperature.

Table 1: buffer preparation

Buffer Name	Concentrated Volume	Amount of Ethanol	Final Volume
GWB	10ml	40ml	50ml
RPB	28ml	12ml	40ml

Procedure of silica-based DNA isolation in quick look



Protocol: Purification of PCR product (based on silica technology)

Sample type: PCR products

Some tips to know:

- All centrifugation steps are carried out at room temperature (15–25°C).
- Not forget to add the appropriate amount of molecular biology grade ethanol to RPB as indicated on the bottle, before using for the first time.
- Not forget to add the appropriate amount of molecular biology grade ethanol (%96–100) to GWB as indicated on the bottle, before using for the first time (refer to buffer preparation section).
- RPB should be pink, if it turns yellow or orange, contact the technical support group.
- If RPB forms precipitate during storage, please warm it to 56°C until the precipitate has fully dissolved.
- RPB should be prepared. Not forget to add the appropriate amount of molecular biology grade ethanol (%96–100) to RPB as indicated on the bottle, before using for the first time (refer to buffer preparation section).

Process

- Transfer PCR product to a clean microcentrifuge tube. Add 5 volume of pre-prepared RPB to the tube.
- Pulse vortex for 15-30s. Transfer the solution to a HiPure DR Column placed in a 2ml collection tube (supplied in the kit box). Centrifuge at 13000 rpm for 1min at room temperature.
- Discard the flow-through. Add 750µl GWB to the spin column. Centrifuge for 1min at 13000 rpm at room temperature. Discard the flow-through, and then centrifuge the HiPure DR Column for an additional 1min at 13000 rpm.

  Note: Discarding the flow-through before the second centrifuge is necessary to remove ethanol.

- Place the HiPure DR Column in a new 1.5ml microcentrifuge tube. Add 30-50µl RSB directly to center of the spin column. Incubate at room temperature (15-25℃) or at 60℃ for 5min. Centrifuge for 1min at 13000 rpm to elute the nucleic acid.

Note: If higher DNA yield is desirable, add another 30 µl RSB directly to the center of spin column. Incubate at room temperature (15-25℃) or at 60℃ for 5min. Centrifuge for 1 min at 13000 rpm to elute the nucleic acid.

Note: Incubating RSB at 60℃ increases the yield, but in some cases, it might lead to the presence of ssDNA.