# Quick Protocol

## Gel and PCR Purification Set

PCR Purification and nucleic acid isolation from gel based on silica technology

- MiniPrep

## Kit Content

| Component | 50 preps | 100 preps |
|---|---|---|
| **RGB** | 30ml | 60ml |
| **RPB** | 28ml | 2 x 28ml |
| **GWB** | 10ml | 2 x 10ml |
| **RSB** | 5ml | 10ml |
| **HiPure DR column** | 50 | 100 |
| **Collection tube** | 50 | 100 |

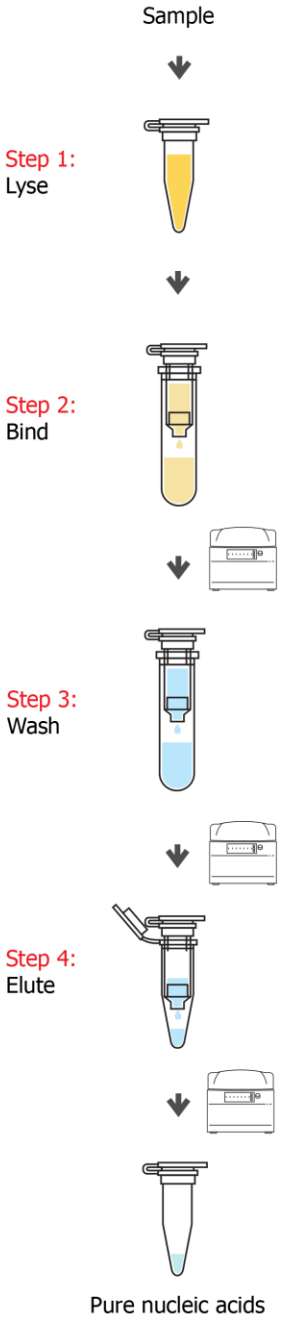
## Buffer Preparation

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 1: Isolation of nucleic acid from agarose gel (based on silica technology)

**Sample type:** DNA bands on agarose gel

### 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 (%96–100) to GWB as indicated on the bottle, before using for the first time (refer to buffer preparation section).
- RGB should be yellow, if it turns pink, set the pH to 5.0 by using $H_2SO_4$.
- If RGB forms precipitate, please warm it to 56°C until the precipitate has fully dissolved.

### Process

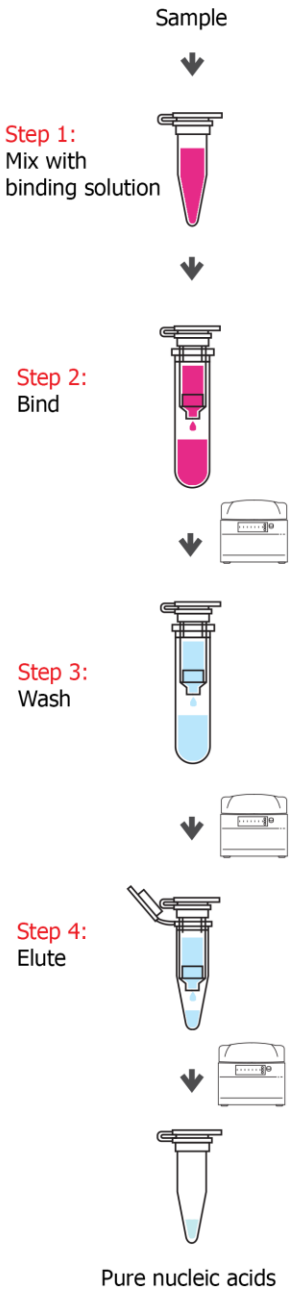
- Remove the DNA fragment from the %1 agarose gel with a clean, sharp scalpel.

  **Note:** removing the extra agarose, and try to minimize the size of the gel slice.
- Weigh the gel slice in a colorless tube. Add appropriate RGB amount to the microcentrifuge tube (for each 100mg of gel, add 300µl RGB. For example, if the gel weight is 200mg add 600µl RGB to the microcentrifuge tube).
- Pulse vortex for 5s and incubate at 50˚C for 10 min. During incubation, pulse vortex every 2min.
- After dissolving the gel completely, add one gel volume of absolute ethanol to the microcentrifuge tube (for example for 30mg gel, add 30µl absolute ethanol), pulse vortex for 15s.
- Transfer the solution to a HiPure DR Column placed in a 2ml collection tube (supplied in the kit box). Centrifuge for 1min at 13000 rpm at room temperature. Discard the flow-through.
- Add 750µl prepared GWB to the spin column.

  **Optional**: For increased DNA concentration, after adding GWB incubate at room temperature (15-25˚C) for 4 min.
- 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.

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

## Procedure of silica-based DNA isolation in quick look



## Protocol 2: 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 30µl RSB directly to the center of 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:** Incubating RSB at 60℃ increases the yield but in some cases, it might lead to the presence of ssDNA.