

# mi-PCR Purification Kit

Cat. No mi-PP200  
[200 Preparations]

This kit is for research purposes only.  
Not for use in diagnostic procedures.  
For in vitro use only.

don't risk your experiment. trust ... *metabion*

## Introduction

The mi-PCR Purification Kit is designed for the rapid purification of PCR products combined with an extraordinary high recovery rate.

The DNA Binding Buffer is added to the PCR reaction and the mixture is applied to a spin column containing silica-based membranes where the double stranded DNA is selectively adsorbed. DNA polymerases, buffer, remaining primers and dNTP are removed with alcohol-containing Column Wash Buffer. Since the DNA is eluted with nuclease free distilled water or TE buffer, no precipitation is necessary. All solutions are passed through the spin column with brief centrifugations, allowing the completion of the protocol in less than 10 minutes. This kit eliminates the use of hazardous chemicals such as phenol or chloroform.

## Specifications

DNA Size range: 100 bp - 15 kb

Necessary time: < 10 min

Recovery rates: up to 95%

Easy protocol: Binding-Washing-Elution

Automated fluorescent sequencing analysis, restriction enzyme analysis, TA cloning, probe labeling, etc.

## Kit Contents

	<b>mi-PCR Purification Kit</b>
Preparation	200 rxn /kit
DNA Binding Buffer	110 ml (store at room temperature)
Column Wash Buffer	50 ml (store at room temperature) Add 200 ml of pure ethanol (99.9%) to the "Column Wash Buffer" up to a final volume of 250 ml prior to first use. (Store at room temperature.)
Spin column	200
Collection tube	200

## Required Equipment

Microcentrifuge (13,000 rpm or 12,000 x g)

Vortexer

Microcentrifuge tubes

Distilled water (pH 7-8) or 10 mM Tris-HCl (pH 8.0)

## Kit Storage

At room temperature for 6 months.

## Precautions

See MSDS on our homepage ([www.mymetabion.com](http://www.mymetabion.com)).

Wear gloves. Avoid contact with all reagents. If eye or skin contact occurs, wash thoroughly with water. Avoid direct contact of DNA Binding Buffer with bleach or other oxidizers. WARNING: The Column Wash Buffer is flammable.

## Protocol

**Note:** Before starting, please make sure....

- To have completed the “Column Wash Buffer” by adding 200 ml of pure ethanol (99,9%) before the first use.
- All centrifugation steps are at 13,000 rpm (or 12,000 x g).
- If the DNA Binding Buffer was precipitated, heat to dissolve at 37°C for 10 minutes.

1. Add 5 volumes of DNA Binding Buffer to the PCR reaction.  
For example, add 500 µl of DNA Binding Buffer to a 100 µl PCR reaction.
2. Mix well by pipetting. If any oil overlay was used, there will be two layers. The top layer is oil. Do not apply the oil to the spin column!
3. Place a spin column into a 2 ml collection Tube. Transfer the PCR/DNA Binding Buffer mixture to a spin column.
4. Spin at 13,000 rpm (or 12,000 x g) for 1 min at room temperature.
5. Remove the spin column and discard the liquid flow-through from the collection tube by decanting.
6. Replace the spin column in the same decanted collection tube.
7. Add 750 µl of Column Wash Buffer to the spin column.  
(Make sure to have completed the “Column Wash Buffer” by adding 200 ml of pure ethanol (99,9%) before first use.)
8. Spin at 13,000 rpm (or 12,000 x g) for 1 min at room temperature.
9. Add 250 µl of Column Wash Buffer to the spin column.
10. Spin at 13,000 rpm (or 12,000 x g) for 1 min at room temperature.
11. Remove the spin column and discard the liquid flow-through by decanting. Then replace spin column back into the same collection tube.
12. To eliminate any possibility of Column Wash Buffer carryover spin at 13,000 rpm (or 12,000 x g) for 1 min at room temperature.
13. Transfer the spin column to a clean microcentrifuge tube (not included).

14. Elute the DNA by adding 50 µl of distilled water or 10 mM Tris-HCl (pH 8.0) solution directly onto the center of the white spin column membrane. The choice of using water or Tris solution at this point will not affect the yield. However, DNA is more stable for storage in Tris buffer. Maximum recovery is obtained with nuclease-free water warmed to 65-75°C.
15. Spin at 13,000 rpm (or 12,000 x g) for 1 min at room temperature.
16. Discard the spin column. The purified DNA is now in the microcentrifuge tube and ready to use. The DNA will be free of all reaction components, such as primers, linkers, enzymes, salt, and dNTPs. Store at -20°C.  
The DNA is now ready to use.

## Hints and Troubleshooting

### Concentrating the DNA

Your final volume will be 50  $\mu$ l. If this is too dilute for your purposes, add 2  $\mu$ l of 5 M NaCl and mix. Then add 100  $\mu$ l of 100% cold ethanol and mix. Spin at 13,000 rpm (or 12,000 x g) for 5 min. Decant all liquid. Dry residual ethanol in a speed vac, desiccator or ambient air. Resuspend precipitated DNA in desired volume.

### Low recovery

- Low recoveries can be due to not mixing the DNA Binding Buffer well with the sample (step 2).
- Incomplete removal of Column Wash Buffer can also reduce yields. Make sure the centrifuge is spinning at a minimum of 13,000 rpm (or 12,000 x g).
- The elution efficiency is dependent on the pH. The maximum elution efficiency is achieved between pH 7.0 and 8.0. When using water, ensure that the pH value is within this range. Store the DNA at -20°C.

### Enzyme reactions inhibited

If you choose to elute the plasmid DNA in a Tris buffer containing EDTA (like TE buffer), you may see inhibition of subsequent enzymatic reactions. Re-purify the sample with this kit and use distilled water (pH 7-8) or Tris solution (10 mM Tris, pH 8.0) for the elution step.