Bio Bharati Life Science Pvt. Ltd.

BBL Gel Extraction & PCR Clean Up Combo Kit
Cat# BB-GPK50 (50 preps)

Introduction:

BBL-Gel Extraction& PCR Clean Up Combo Kit is a column based nucleic acid purification kit where the column has silica membrane which is capable of purifying DNA fragments easily from TAE or TBE agarose gel (both normal and low melting point agarose) or PCR.

This kit can purify DNA fragments of 80 bp to 10Kbp with a recovery rate of more than 75%. The purified DNA is suitable for basic molecular biology experiments like restriction digestion, ligation and PCR amplification.

Kit Contents:

Contents	# BB-GPK50 (50 preps)
Binding Buffer	25 ml
Wash Buffer	15 ml
Elution Buffer	15 ml
Spin Column	50 nos
Collection Tubes 2.0 ml	50 nos

Storage:

BBL-Gel Extraction & PCR Clean up Combo Kit (**# BB-GPK50)** is stable for 12 months if stored properly in a dry place at room temperature (20°C - 25°C) not exposed to sunlight.

Note:

- ✓ Add ethanol (96-100%) to Wash buffer before use (see bottle label for volume)
- ✓ All centrifugation steps are carried out at 12000 rpm (~13400xg) in a conventional table top centrifuge at room temperature (15-25°C).
- ✓ Ensure that the elution buffer is dispensed directly onto the membrane for complete elution of bound DNA. The average volume of elution buffer is ~50 µl. Less volume of elution buffer may lead to lower recoveries of DNA.
- ✓

Protocol

A. DNA Gel Extraction

1. **Excise the desired DNA fragment from the agarose gel with a clean, sharp scalpel.**
   Trim the gel slice to remove extra agarose. Higher UV wave length & shorten exposure of gel during excision will reduce UV-induced DNA damage.

2. **Weigh the gel slice in a clean colourless micro-centrifuge tube. Add 1 gel volume of Binding Buffer to the tube. (100 mg ~ 100 µl).**

3. **Incubate at 55° for 5-10 minutes (or until the gel slice dissolves completely). Mix by inverting tube intermittently to facilitate the process.**
   For >2% gels, increase the incubation time.
   NOTE: **If the target DNA fragment size is <300 bp, add ½ gel volume of Isopropanal to the sample and mix**

4. **Place a spin column in a 2 ml collection tube provided in the kit.**

5. **To bind DNA, apply the sample, to the spin column. Keep at room temperature for 2 min for binding and then centrifuge for 1 min**
   The maximum volume of the column reservoir is 750 µl. For sample volumes of more than 750 µl, repeat the step 5 again.

6. **Discard the flow through and place the spin column back in the same collection tube.**

7. **Add 600 µl of Wash Buffer to the column and centrifuge for 1 min. Discard the flow through and place the spin column into the collection tube.**

8. **Repeat the step 7 for one more time.**

9. **Discard the flow through and centrifuge the tube for an additional 1 min**
   Residual ethanol from Wash Buffer will not be completely removed unless the flow through is discarded before this centrifugation.

10. **Place the spin column into a fresh, clean 1.5 ml micro centrifuge tube.**

11. **To elute DNA, add 50 µl of Elution Buffer to the centre of the membrane. Incubate at room temperature for 3-5 minutes and then centrifuge for 2 min.**

12. **Take 2-5 µl purified DNA for concentration check and electrophoresis and store the rest of the DNA at -20°C for further analysis.**

B. PCR Purification

1. **Add 4 volumes of Binding Buffer to 1 volume of the PCR sample and mix. (Removal of mineral oil from the top is not necessary).**
   For example, add 400 µl of Binding Buffer to 100 µl of PCR sample (not including oil)

2. **Place a spin column in a 2 ml collection tube provided in the kit.**

3. **To bind DNA, apply the sample mix, to the spin column. Keep at room temperature for 2 min for binding and centrifuge for 1 min**

4. ***The maximum volume of the column reservoir is 750 µl. For sample volumes of more than 750 µl, repeat the step 3 again.***

5. **Discard the flow through and place the spin column back in the same collection tube.**

6. **Add 600 µl of Wash Buffer to the column and centrifuge for 1 min. Discard the flow through and place the spin column into the collection tube.**

7. **Repeat the above step 5 for one more time.**

8. **Discard the flow through and centrifuge the tube for an additional 1 min**

9. ***Residual ethanol from Wash Buffer will not be completely removed unless the flow through is discarded before this centrifugation*.**

10. **Place the spin column into a fresh, clean 1.5 ml micro centrifuge tube.**

11. **To elute DNA, add 50 µl of Elution Buffer to the centre of the membrane. Incubate at room temperature for 3-5 minutes and then centrifuge for 2 min.**

12. **Take 2-5 µl purified DNA for concentration check and electrophoresis and store the rest of the DNA at -20°C for further analysis.**