Truin Science

PCR Cleanup Kit

(50) Cat. # KTS1100
(100) Cat. # KTS1115

Contents:	**50 Preps**	**100 Preps**
Binding Buffer **(BD)**	(20 ml)	(40 ml)
Wash Buffer **(PE)**	(15 ml)	(2 x 15 ml)
Elution Buffer **(EB)** (10mM Tris-HCl, pH 8.5)	(2.5 ml)	(5 ml)
Spin Columns	(50 each)	(100 each)

- **Prior to use, add 60ml of ethanol (95-100% not provided) to Wash Buffer PE.**

Protocol

1. Transfer the PCR reaction mix or other enzymatic reaction mix to new 1.5ml microcentrifuge tube. Add a 1:1 volume of Binding Buffer **BD** to the mixture (e.g. for every 100 µl of reaction mix, add 100 µl of Binding Buffer **BD**).

2. Transfer up to 800 µl of the solution from step 1 to the spin column. Incubate for 2 min at room temperature.

3. Centrifuge for 1 min at 12,000 rpm. Discard-flow through.
Note. *If the total volume exceeds 800 µl, the solution can be added to the column in stages. After the addition of 800 µl of solution, centrifuge the column for 30-60 seconds and discard flow-through. Repeat until the entire solution has been added.*

4. Add 500 µl of Wash Buffer **PE** to the column. Centrifuge for 1 min at 12,000 rpm. Discard flow-through and place the purification column back into the collection tube.
Note. Wash Buffer PE must previously be diluted with 60 ml ethanol (95-100%).

5. Repeat step 4.

6. Centrifuge the empty column for an additional 3 min to completely remove any residual wash buffer.
Note. *This step is essential as the presence of residual ethanol in the DNA sample may reduce yields and may inhibit subsequent enzymatic reactions.*

7. Empty the collection tube and centrifuge again for 1 min with the microcentrifuge lid open (or removed) to allow evaporation of residual ethanol.

8. Place the spin column in a clean 1.5ml microcentrifuge tube (not provided), and pipette 50 µl (30-100 µl) of Elution Buffer **EB** directly to the center of the column without touching the membrane. Incubate at room temperature for 2 min.
Note. *To increase yield of large DNA, warm the Elution Buffer to 60°C. For low DNA amounts the elution volumes can be reduced to increase the final DNA concentration. An elution volume between 20-50 µl does not significantly reduce the DNA yield. However, elution volumes less than 10 µl are not recommended.*

9. Centrifuge for 1 min at 12,000 rpm. Discard the columns and store the microcentrifuge tube containing the eluted DNA at -20°C.