DANAGENE CLEAN PCR KIT

REF.0501.1 50 PURIFICATIONS
REF.0501.2 250 PURIFICATIONS
REF.0501.3 1000 PURIFICATIONS

1.INTRODUCCION

DANAGENE CLEAN PCR KIT is a versatile and effective method **for a quick PCR products clean up**. This method allows the purification of DNA up to 15 μg.

The purification is based on the selective absorption of nucleic acids in spin mini-columns with silica membranes with chaotropic agents presence.

The purified DNA is eluted in a small buffer volume (5 mM Tris-HCl pH 8.5) and can be used in enzymatic manipulations, including sequencing, cloning, restriction analysis, ligations, labelling and *in vitro* transcription.

DANAGENE CLEAN PCR KIT allows a DNA recovering of .01-10 Kb with an efficiency that varies between 75-90% depending on the fragment size.

2. KIT COMPONENTS

	Ref. 0501.1 50 preps	**Ref. 0501.2 250 preps**	**Ref. 0501.3 1000 preps**	
Binding Solution	15 ml	60 ml	240 ml	Room temperature
Wash Solution	10 ml	50 ml	200 ml	Room temperature
Elution Solution	2 ml	10 ml	40 ml	Room temperature
Spin Columns	50 unid.	250 unid.	1000 unid.	Room temperature
Recollection Tubes	50 unid.	250 unid.	1000 unid.	Room temperature

Equipment and aditional reagents required
* Microtubes.
* Microcentrifuge.
* Water bath.
* Ethanol 100 %.
* Isopropanol.

3. PROTOCOL

The protocol implies the following steps:

- **DNA binding**: the sample that has to be purified goes through the Spin column and this promove the DNA binding to the silica membrane.
- **DNA purification:** washing with the wash solution that contains ethanol and removes contaminants, oligos, proteins, etc.
- **DNA recovering:** the DNA is eluted with 5 mM Tris-HCl pH 8.5 or sterile water pH 8.5.

3.1 Preparaciones preliminares

• **ADD 40 ml** 50 test**, 200 ml** 250 test o **800 ml** 1000 test **OF 100% ETHANOL TO THE WASH SOLUTION**
Label the container and keep it closed to avoid ethanol evaporation.

• **ADD 10 ml** 50 test , **40 ml** 250 test o **160 ml** 1000 test **OF 100% ISOPROPANOL TO THE BINDING SOLUTION**
Label the container and keep it closed to avoid isopropopanol evaporation.

• Pre-heat the Elution Buffer at 70ºC.

3.2 Protocol

1. **Add 4 volumes Binding Solution with isopropanol** to 1 volume of PCR (50 -100 µl). For example, 50 µl PCR mix + 200 µl Binding buffer with isopropanol. Mix well by pipetting.

2. **Transfer the sample to a spin column** with your recollection tube.

3. **Centrifuge for 1 minute at 10.000-12.000 r.p.m.**

4. **Remove the filtrate and add 600 µl of the Washing solution** . Centrifuge for 1 minute at 14.000 r.p.m.

5. **Remove the filtrate and add 200 µl of washing solution.** Centrifuge for 1 minute at 14.000 r.p.m.

6. **Remove the residual ethanol** by centrifugation for 3 minutes at 14.000 r.p.m.

7. Transfer the spin column into a new receiver tube and add **at least 25 µl of pre-warmed Elution Buffer** a**t 70ºC.** A second step of elution will increase performance.

8. **Incubate for 2 minutes and centrifuge for 1 minute at 14.000 r.p.m.**

4. PROBLEM GUIDE AND POSSIBLE ANSWER

For any doubts or additional questions about the protocol, please contact the technical service of DANAGEN-BIOTED S.L info@danagen.es