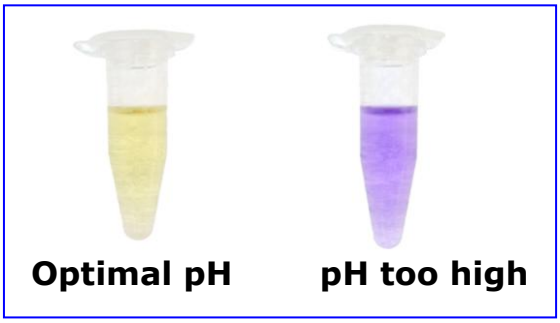
DANAGENE GEL / PCR KIT

REF.0502.1 50 PURIFICATIONS
REF.0502.2 250 PURIFICATIONS
REF.0502.3 1000 PURIFICATIONS

1. INTRODUCTION

The **DANAGENE GEL/PCR SPIN Kit** was designed to recover or concentrate **DNA fragments from agarose gel, PCR or other enzymatic reactions.**

This **kit** includes a pH indicator which is premixed with the binding buffer to ensure optimal pH, facilitate DNA binding and allow for easy observation of undissolved agarose gel. If pH exceeds the optimal level (>7.5), the color of the solution will appear purple instead of yellow. 3M Sodium Acetate (pH5.0), which is included with the kit, can then be added to the solution to adjust pH and return the color to yellow.



Features:

- **High Efficiency: up to 90% recovery from agarose gels, up to 95% recovery from PCR or other enzymatic reactions.**
- **Sample Size: up to 300 mg of agarose gel slice or up to 100 µl of PCR products.**
- **Broad Fragment Size Range: 70 bp–20 kb.**
- **Primers and primer/dimer removal.**
- **The protocol is done in 10 minutes.**

2. COMPONENTS KIT

	Ref. 0502.1 50 preps	Ref. 0502.2 250 preps	Ref.0502.3 1000 preps	T[a]
Solubilization and Binding Buffer	30 ml	150 ml	4 x 150 ml	RT
3M Sodium Acetate (pH 5.0)	500 µl	500 µl	4 x 500 µl	RT
Wash Solution	10 ml	50 ml	4 x 50 ml	RT
Elution Buffer	2 ml	10 ml	4 x 10 ml	RT
Spin Columns	50 unid.	250 unid.	1000 unid.	RT
Collection Tubes	50 unid.	250 unid.	1000 unid.	RT

Equipment and additional reagents required

** 100 % Ethanol.*
** 1.5 ml. microtubes*
** Microcentrifuge.*
** Water Bath.*

3. PROTOCOL

3.1 Preliminary Preparations

- *Add **40 ml** (50 test) or **200 ml** (250 test) of **100% Ethanol to the wash solution** .Label the container and keep it closed to avoid ethanol evaporation.*
- *Pre-heat the Elution Buffer at 70ºC.*

3.2 Protocol 1:

✓ for routine purifications of **70 bp - 20 Kb dsDNA PCR fragments** from 10-40 mer primers, dNTPs, enzymes and salts mixtures.

1. **Add 5 volumes of Solubilization and Binding Buffer to 1 volume of PCR (50 -100 µl).** Mix well. If the mixture has turned from yellow to purple, add **10 µl** 3M Sodium Acetate (pH 5.0) and mix thoroughly.This will adjust pH and the color return to yellow.

2. **Transfer the sample to a spin column.** Put the spin column in collecting tube.

3. **Centrifuge for 1 minute at 10.000-12.000 rpm.**

4. **Remove the filtrate and use 600 µl of the Washing solution**. Centrifuge for 1 minute at 14.000 rpm.

5. **Remove the filtrate and apply 200 µl of washing solution.** Centrifuge for 1 minute at 14.000 rpm.

6. **Remove the residual ethanol** by centrifugation for 3 minutes at 14.000 rpm.

7. Transfer the spin column into a new receiver tube and add **at least 25 µl of pre-warmed Elution Buffer** a**t 70ºC.**

8. **Incubate for 2 minutes and centrifuge for 1 minute at 14.000 rpm.**

3.3 Protocol 2:

- ✓ **for solubilization of the agarose gel slice for extractions of DNA fragments**

1. To purify agarose gels, cut the band and **try to minimize the fragment size** removing the excess of agarose. **Transfer up to 300 mg of gel slice** to a 1.5 ml microcentrifuge tube.

   Note:*If* *using less than 300 mg of gel slice,the buffer does not to be scaled. If using more than 300 mg of gel slice, separate it into multiple 1.5 ml microcentrifuge tube.*

2. **Add 500 µl of Solubilization and Binding Solution** .

   Note: If the mixture has turned from yellow to purple, add **10 µl** 3M Sodium Acetate (pH 5.0) and mix thoroughly.This will adjust pH and the color return to yellow.

3. **Incubate for 10 minutes at 55ºC** to dissolve the agarose. **Shake** with a vortex every 2-3 minutes.

4. **Transfer the solution to a spin column.** Put the spin column in collecting tube.

5. **Centrifuge for 1 minute at 10.000-12.000 rpm.**

6. **Remove the filtrate and use 600 µl of the Washing solution** (ref. EP08). Centrifuge for 1 minute at 14.000 rpm.

7. **Remove the filtrate and apply 400 µl of washing solution.** Centrifuge for 1 minute at 14.000 rpm.

8. **Remove the residual ethanol** by centrifugation for 3 minutes at 14.000 rpm.

9. Transfer the spin column into a new receiver tube and add **at least 25 µl of pre-warmed Elution Buffer at 70ºC.**

10. **Incubate for 2 minutes and centrifuge for 1 minute at 14.000 rpm.**

4. PROBLEM GUIDE AND POSSIBLE ANSWER

For any question regarding the work protocols o problems. Please, contact DanaGen-BioTed technical service for any comment or question regarding the protocol.