Component Storage Requirements:

Component	Conditions for Storage
PCR Spin Tubes	RT storage, indefinite storage time
Binding Buffer (Buffer #1)	Store above 22°C, protect from light. Expiration date as stated on label.
Wash Buffer (Buffer #2)	RT storage, expiration date as stated on label
Elution Buffer (Buffer #3)	RT storage, expiration date as stated on label.
Wash Tubes	RT storage, indefinite storage time
Sample Collection Tubes	RT storage, indefinite storage time

Product Description	Catalog Number
PSIΨClone Single Tube kit (Spin tubes, wash tubes, sample collection tubes & buffers for 75 preps)	PC-511
PCR Binding Buffer (60 mL bottle)	PC-503

79-100820A

PRINCETON SEPARATIONS

P.O. Box 300
Adelphia, NJ 07710
800.223.0902
e-mail: info@prinsep.com
www.prinsep.com

PSIΨClone PCR ST

A kit for purification of amplification products from PCR reactions.

Centrifugation Protocol.

This **PSIΨClone PCR ST** (single tube) kit is designed to provide the researcher with a rapid method for processing PCR reaction products in a convenient single tube format for centrifugal application. The protocol is fast and results in high yield, high purity DNA which is suitable for use in molecular biology procedures.

DNA fragments (ranging from 100 bp to 10 kb) from PCR reactions bind to the membrane in the spin tube supplied with the kit. Subsequent wash steps remove residual primers, nucleotides, enzymes, and salts. Following wash steps, DNA is eluted in a low salt buffer in high yield (Table 1) and is suitable for further molecular operations without additional processing.

Residual primers, nucleotides, enzymes and salts in PCR amplification reaction products may interfere with molecular operations (e.g. cloning, sequencing etc.). The **PSIΨClone PCR ST** kit efficiently removes these residual reactants using a convenient single tube format. The purified amplicons are ready for direct sequencing or subcloning.

Processing of amplification products from PCR reactions using centrifugation.

Add 48 mL ethanol (95-100%) to the Wash Buffer concentrate (#2) prior to use.

Kit Components and Preparation (see back page for storage conditions):

PCR Spin Tubes: The spin tubes are supplied ready to use.

Binding Buffer: This solution is subject to crystallization at temperatures below 22°C. If crystals form, warm the buffer to 50°C to dissolve.

Wash Buffer: Provided as a concentrate, the Wash Buffer requires dilution with ethanol to a final ethanol concentration of 75-80%. See instructions above

Elution Buffer: Use as provided.

Wash Tubes: Provided for use with binding and wash steps.

Sample Collection Tubes: Provided for use with final elution step.

The PSI Clone PCR DNA purification kit provides sufficient materials to perform 75 separate DNA purifications from standard PCR reactions.

Protocol:

1. Add 1 volume of PCR binding buffer[1,2] to 1 volume of PCR reaction[1,2] product and mix thoroughly (vortex).
2. Put a single PCR column in a wash tube (2 mL) and transfer the DNA mixture to the PCR column (filter) and centrifuge at 8,000-x g for 30-60 seconds. Discard the flow thru.
3. Add 400 µL PCR wash buffer (diluted with ethanol) to the PCR column and centrifuge a 8,000-x g for 30-60 seconds. Discard the flow thru.
4. Repeat step 3 once more. Ensure that the membrane is spun dry.
   a. (optional) re-spin (as in step 4-above) the PCR column empty to remove residual wash buffer.
5. Add 50-65 µL PCR elution buffer and incubate 2-5 minutes to allow buffer to soak into the membrane.
6. Place the PCR column in a DNAse free (autoclaved) collection tube and centrifuge at 8,000 x g for 30-60 seconds to collect the eluant. Discard the PCR column and save the flow thru (eluant).

Notes:

1. Follow this protocol for amplicons in the range of 150 bp to 10 kb.
2. For fragments smaller than 150 bp and larger than 70 bp, revise step 1 of the protocol. Use 5 volumes of PCR binding buffer to 1 volume of PCR reaction product (Table 2). Additional binding buffer will be required and is available as Catalog Number PC-503 (60 mL bottle of binding buffer).

Materials Required but not Supplied:

Either 95% ethanol or absolute ethanol.
Centrifuge.

Table 1. Average recovery for DNA fragments of various lengths.

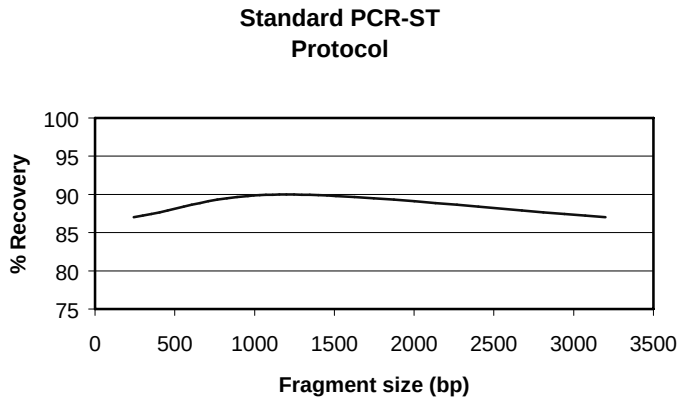
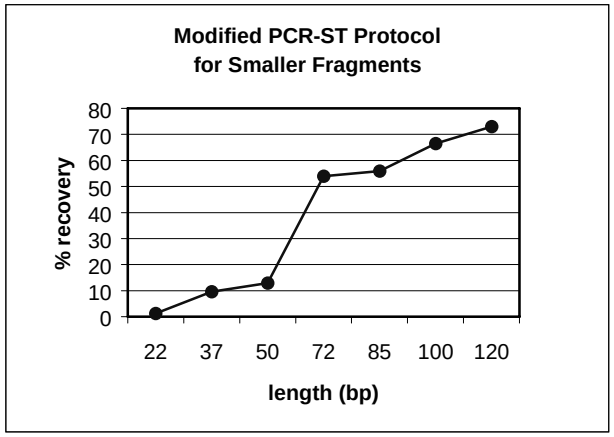


Table 2. Average recovery for small fragments using modified protocol.



Troubleshooting:

Failure to follow the protocol at the wash step may result in low recovery of fragments.

- Check that ethanol was added to the Wash Buffer (#2).
- Thoroughly dry the spin tubes before adding the Elution Buffer.