Push Column Beta Shield Device and NucTrap® Probe Purification Columns

INSTRUCTION MANUAL

Catalog #400700 (Push Column Beta Shield Device), #400701 (25 columns) and #400702 (50 columns)

IN #70024-07

Revision #115005

400701-12 115005/

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Push Column Beta Shield Device and NucTrap® Probe Purification Columns

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MATERIALS PROVIDED

Push Column Beta Shield Device (Catalog #400700)

Caution *Stratagene recommends using the Push Column Beta Shield Device in conjunction with the NucTrap probe purification columns to protect the user from radiation. The Push Column Beta Shield Device is designed specifically to support the NucTrap probe purification columns while in use. If the Push Column Beta Shield Device is not used, proper shielding and caution should be exercised when separating radiolabeled free nucleotides using the NucTrap probe purification columns.*

Material Provided	Catalog #400700
Beta shield base	1
Column beta shield with a column-locking mechanism	1
Syringe cover	1

NucTrap Probe Purification Columns (Catalog #400701 and #400702)

Note *If the column resin appears displaced, dry, or cracked, rehydrate the column with 80 µl of the appropriate buffer (i.e., 1 × STE buffer for radiolabeled probes or 1 × STET buffer for biotinylated probes) prior to equilibrating the column. The column is functional following rehydration, although the resin may still appear cracked.*

	Catalog #	
Material provided	400701	400702
NucTrap® probe purification columns[a]	25	50
B-D® syringes with Luer-Lok® tips	10	20
Crocein Orange G Dye	1	1
Blue Dextran Dye	1	1

[a] Store the NucTrap probe purification columns at room temperature.

Storage Conditions

All Components: Room temperature

IN #70024-07/Revision #115005

INTRODUCTION

Stratagene's NucTrap® probe purification columns* rapidly separate unincorporated nucleotides from radiolabeled DNA or RNA probes. The NucTrap probe purification columns can isolate DNA or RNA probes as small as 17 bp and as large as 50,000 bp from unincorporated nucleotides in less than 2 minutes.

To protect the user against radiation during operation of the NucTrap probe purification columns, Stratagene offers the Push Column Beta Shield Device separately from the columns. This three-piece acrylic device separates the sample by applying pressure on a syringe screwed onto a column that is placed within the column beta shield. The DNA–RNA solution flushes through the resin in seconds, yielding probes and/or oligonucleotides in a volume of ~150 µl or less.

* U.S. Patent Nos. 5,336,412 and 5,378,360.

PRE-OPERATING INSTRUCTIONS

Dye Sample Test

The following two nontoxic dyes are included to provide a safe, visual evaluation of the effectiveness of the columns: Blue Dextran, a 2×10^6-molecular-weight marker, and Crocein Orange G, a 350.3-molecular-weight marker.

1. Prepare the dye sample by adding 80 µl of the Crocein Orange G to 80 µl of the Blue Dextran. Mix the dye sample well.

2. Remove the light blue caps from a column.

3. Equilibrate the column as outlined below:

    a. Load 80 µl of 1× STE buffer (see *Preparation of Media and Reagents*) to the resin at the top of the column.

    b. Extend the plunger of a 10-cm^3 B-D® syringe with a Luer-Lok® tip and then screw the syringe onto the column.

    c. Push the plunger down and allow the STE buffer to flow through the column until the buffer reaches the bottom of the resin.

    d. Unscrew the syringe and remove the syringe from the column.

4. Load 80 µl of the mixed dye sample to the resin at the top of the column.

5. Screw the syringe with the plunger extended onto the column.

6. Push the plunger down and collect the eluate in a 1.5-ml microcentrifuge collection tube.

    Note *The viscosity of the mixed dye sample is greater than a normal aqueous DNA sample, increasing the resistance by which the dye sample flows through the column. Therefore, the dye sample test takes ~2 minutes to complete as opposed to ~1 minute for a normal DNA sample.*

7. Unscrew the syringe and repeat steps 3a–3d with 80 µl of 1× STE buffer.

The Blue Dextran (representing the probe) flows through the column, while the Crocein Orange G (representing the free nucleotides) remains at the top of the column. This separation results from the size difference of the molecules and is independent of charge.

OPERATING INSTRUCTIONS

Setting up the Push Column Beta Shield Device

Figure 1 provides a schematic diagram for setting up the Push Column Beta Shield Device.

1. Remove the syringe cover.

2. Remove the column beta shield from the beta shield base.

3. Insert a 1.5-ml microcentrifuge collection tube into the hole in the bottom of the beta shield base.

Preparing the NucTrap Probe Purification Column

1. Remove the light blue caps from the column.

   Note *If the column resin appears displaced, dry, or cracked, rehydrate the column with 80 µl of the appropriate buffer (i.e., 1 × STE buffer for radiolabeled probes or 1 × STET buffer for biotinylated probes) prior to equilibrating the column. The column is functional following rehydration, although the resin may still appear cracked.*

2. Equilibrate the column by loading 80 µl of 1× STE buffer to the resin on top of the column.

3. Extend the plunger of a 10-cm^3 B-D syringe with a Luer-Lok tip and screw the syringe onto the column snugly to form a seal between the column and the syringe. **Do not screw the syringe on too tight.**

4. Force the buffer down the length of the column until a small drop exits at the end (Figure 2A). The user should be able to see the resin wetting as the buffer travels the length of the column. For best results, use the prewetted column within 5–10 minutes.

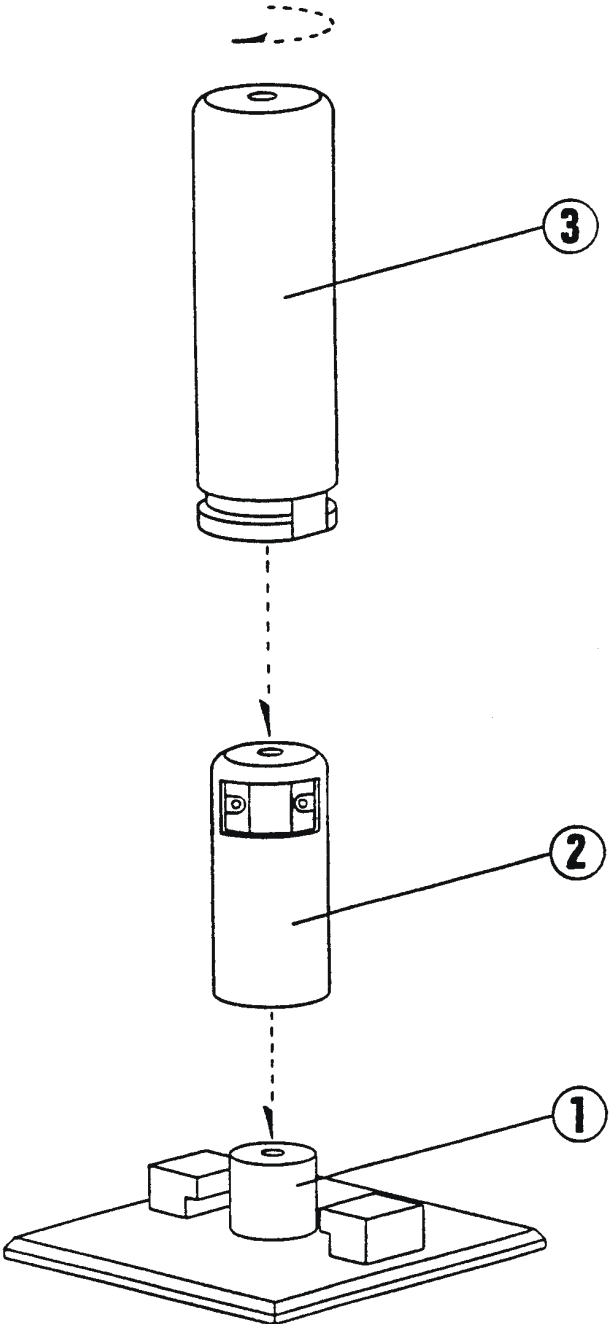

FIGURE 1 Schematic diagram for setting up the Push Column Beta Shield Device. (1) Beta shield base; (2) column beta shield with the column-locking mechanism; (3) syringe cover.

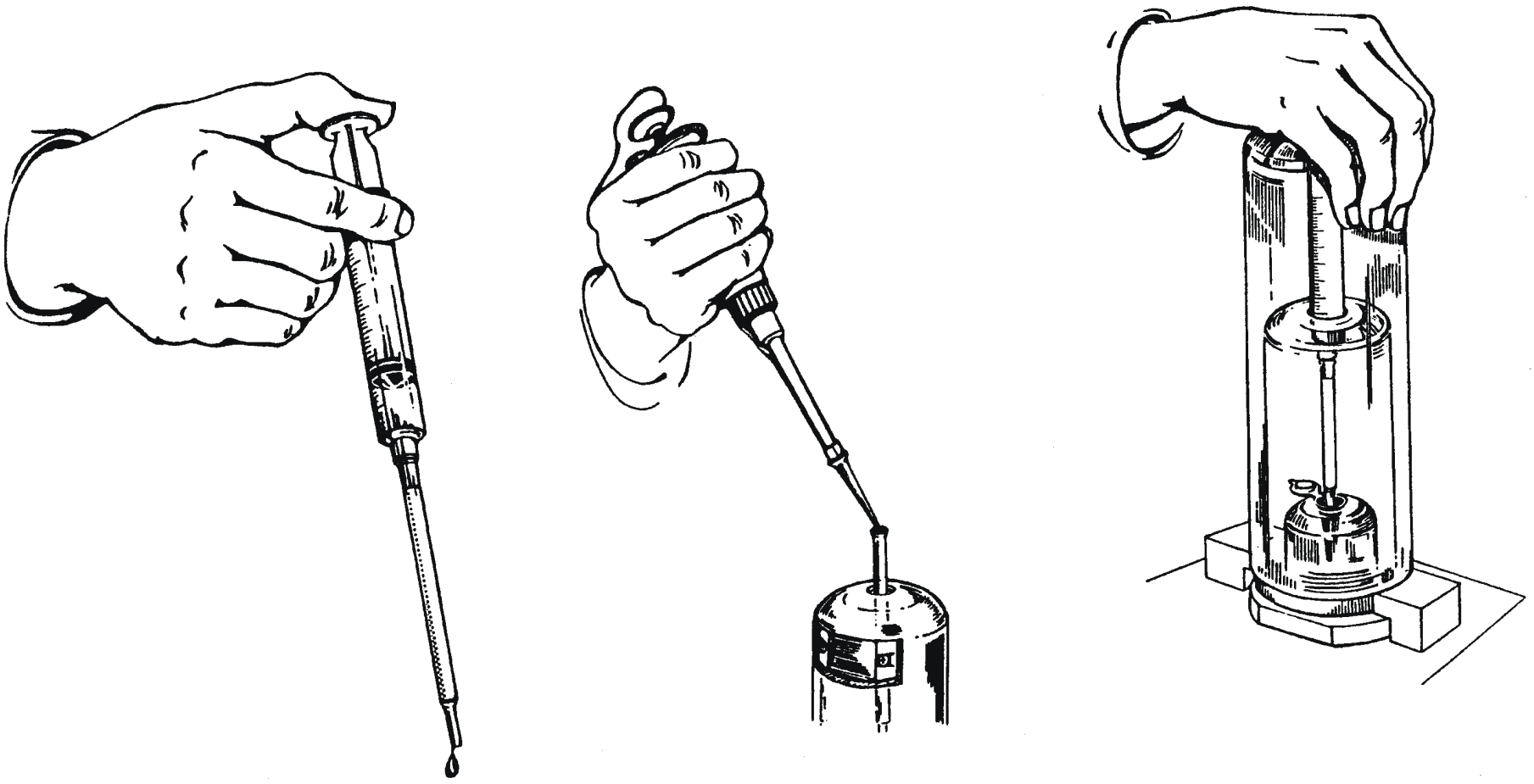

FIGURE 2 Schematic diagram for preparing the columns and for loading and separating the sample using the NucTrap probe purification columns and Push Column Beta Shield Device. (A) Prewetting the column. (B) Loading the sample onto the resin at the top of the column. (C) Pushing the sample through the column.

Assembling the Push Column Beta Shield Device and the NucTrap Probe Purification Column

1. Push the column-locking mechanism on the column beta shield to open and insert a column three-fourths of the way into the mechanism.

2. Release the column-locking mechanism to firmly lock the column in place.

3. Place the column beta shield with the attached column on the beta shield base. The end of the column should empty into a microcentrifuge collection tube set into the hole in the beta shield base.

Loading and Separating the Sample

1. Load 80 µl of the sample onto the resin at the top of the column (Figure 2B).

   Note *Do **not** load a sample with >0.15% (w/v) sodium dodecyl sulfate (SDS), since SDS will expand the resin matrix and the column will not perform optimally.*

2. Extend the plunger on the syringe.

   Note *The plunger of the syringe must be extended **before** the syringe is screwed onto the column. Otherwise, the resin and the sample will be vacuumed into the syringe.*

3. Screw the syringe onto the column.

4. Push the column-locking mechanism and move the column down until the syringe fits snugly against the column beta shield.

   Note *The plunger must remain extended.*

5. Make sure the end of the column is in the top of the microcentrifuge collection tube. Then place the syringe cover over the extended plunger of the syringe.

6. Using direct pressure, **slowly** push down on the syringe cover (Figure 2C), forcing the sample through the column and into the microcentrifuge collection tube. This process should take from ~25 to 35 seconds.

   Note *Force the plunger down at a slow and constant rate, **not** all at once. Please note that the syringe cover **will not lock** into the beta shield base during sample separation. The syringe cover, however, should always be locked into the beta shield base while the Push Column Beta Shield Device is being transported.*

7. Remove the syringe cover and unscrew the syringe from the top of the column. Monitor the syringe for radioactivity. While keeping the column in the column beta shield, rinse the column by loading 80 µl of 1× STE buffer on the top of the column and by pushing this rinse through the column as described previously in step 6. The liquid from the sample and the rinse are now combined. The separation process is complete when liquid no longer drips from the bottom of the column (25–30 seconds). For maximum probe yields, a bolus of air can be pushed through the rinsed column to dislodge any remaining liquid. Unincorporated small molecules remain trapped in the column resin.

   Note *NucTrap probe purification columns are packed and shipped dry. Therefore, a recovery volume of about 10–20 µl less than the starting volume (sample + rinse) can be expected.*

Biotinylated Probes

Biotinylated probes can also be removed using a NucTrap probe purification column. Follow the procedure above, noting the modifications outlined below.

- Following the labeling reaction, add 5 µl of 1% Tween® 20 and 15 µl of 1× STET buffer (see *Reagent Preparation*) to the labeled sample. The final sample volume at this point should be 80 µl.
- To prewet the column, use 80 µl of **1× STET buffer** instead of **1× STE buffer**.
- Load the sample according to the protocol (see *Loading and Separating the Sample*).
- Rinse the column with **1× STET buffer** instead of **1× STE buffer**.

> ***Note*** *As indicated in step 7 of* Loading and Separating the Sample, *the liquid resulting from application of both the sample and the rinse to the column should be collected in one microcentrifuge tube. The final volume of the liquid in the microcentrifuge collection tube should be ~140* µl.

At this point, the biotinylated probe should be separated completely from the unincorporated nucleotides. The probe concentration should be ~50 ng/140 µl or 0.35 ng/µl, assuming 100% recovery of the probe from the column.

Disposal

1. Remove the syringe from the column and monitor the syringe for radioactivity.
2. To remove the column, press the column-locking mechanism and allow the column to drop into a radioactive waste container.
3. Monitor the beta shield device for radioactivity.

TROUBLESHOOTING

Observation	Suggestion(s)
The column resin appears displaced, dry, or cracked prior to use.	Prior to equilibrating the column, rehydrate the column with 80 µl of the appropriate buffer (i.e., 1 × STE buffer for radiolabeled probes or 1 × STET buffer for biotinylated probes). The column is functional following rehydration, although the resin may still appear cracked.
The plunger of the syringe is pushed down, but liquid is not draining out of the bottom of the column into the microcentrifuge collection tube.	Make sure that the column is properly sealed with the syringe.
Absence of spray following application of the sample to the column.	Liquid exiting the column should progress from round, well-formed drops at the beginning of a push to a splitting spray toward the end of a push. This progression indicates the gradual breaking of the liquid seal at the bottom of the column. If this spray is not observed, Stratagene recommends pushing a bolus of air through the column to ensure removal of the remaining liquid.
The column-locking mechanism sticks.	Lubricate the column-locking mechanism with a Teflon® lubricant such as TRI-FLON®.

PREPARATION OF MEDIA AND REAGENTS

1× STE Buffer

100 mM NaCl
20 mM Tris-HCl (pH 7.5)
10 mM EDTA

1× STET Buffer

100 mM NaCl
20 mM Tris-HCl (pH 7.5)
10 mM EDTA
0.1% of Tween 20

ENDNOTES

PUSH COLUMN BETA SHIELD DEVICE AND NUCTRAP® PROBE PURIFICATION COLUMNS

Catalog #400700, #400701, and 400702

QUICK-REFERENCE PROTOCOL FOR RADIOLABELED PROBES

- **Optional:** Evaluate the NucTrap® probe purification columns using the dyes provided (Blue Dextran and Crocein Orange G).
- Set up the push column beta shield device; see Figure 1
- Equilibrate the column by applying 80 µl of 1× STE buffer (no sample)
- Lock the equilibrated column into the beta shield device
- Bring the sample to a final volume of 80 µl using 1× STE buffer
- Screw the syringe (with plunger extended) onto the column and lower the whole assembly into the beta shield device so that (1) the syringe is resting snugly in the beta shield device and (2) the end of the column is placed to elute inside the collection microcentrifuge tube
- Push down with slow, even pressure on the syringe cover to push the sample through the column and into the collection tube
- Remove the syringe from the column
- Apply 80 µl of 1× STE buffer to the top of the column and elute into the same collection tube as before; this ensures complete recovery of the sample into a single collection tube

  Note *The total volume collected will be between 100 and 140 µl because the column resin will retain a small amount of liquid.*

- **Optional:** A bolus of air can be pushed through the column to maximize liquid removal
- Dispose of the radioactive column according to the safety regulations at your facility

PUSH COLUMN BETA SHIELD DEVICE AND NUCTRAP® PROBE PURIFICATION COLUMNS

Catalog #400700, #400701, and 400702

QUICK-REFERENCE PROTOCOL FOR BIOTINYLATED PROBES

- **Optional:** Evaluate the NucTrap® probe purification columns using the dyes provided (Blue Dextran and Crocein Orange G).
- Set up the push column beta shield device; see Figure 1
- Equilibrate the column by applying 80 µl of 1× STET buffer (no sample)
- Lock the equilibrated column into the beta shield device
- To the biotinylated sample add 5 µl of 1% Tween® 20 and 1× STET buffer so that the final volume is 80 µl
- Screw the syringe (with plunger extended) onto the column and lower the whole assembly into the beta shield device so that (1) the syringe is resting snugly in the beta shield device and (2) the end of the column is placed to elute inside the collection microcentrifuge tube
- Push down with slow, even pressure on the syringe cover to push the sample through the column and into the collection tube
- Remove the syringe from the column
- Apply 80 µl of 1× STET buffer to the top of the column and elute into the same collection tube as before; this ensures complete recovery of the sample into a single collection tube

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