

ChipShot™ Indirect Labeling and Clean-Up System

INSTRUCTIONS FOR USE OF PRODUCTS Z4000

Quick
PROTOCOL

cDNA Synthesis from Total or mRNA

cDNA Synthesis from Total RNA

1. Assemble the following in a microcentrifuge tube, keeping the reagents on ice.

Total RNA or Total RNA Positive Control	5µg
Random Primers (3µg/µl)	1µl
Oligo(dT) Primer (2µg/µl)	1µl
Nuclease-Free Water to a volume of	20µl

2. Incubate the solution at 70°C for 10 minutes, then place on ice.
3. Prepare the extension mix as follows:

Component

ChipShot™ RT 5X Buffer	8.0µl
MgCl ₂ (25mM)	4.8µl
aminoallyl-dNTP mix	4.0µl
ChipShot™ Reverse Transcriptase	3.2µl
Nuclease-Free Water to a final volume of	20µl

4. Add the entire 20µl extension mix to each RNA/primer solution, vortex, spin briefly, and incubate at 22–25°C for 10 minutes.
5. Incubate at 42°C for 2 hours, protected from light. Proceed to RNase treatment.

cDNA Synthesis from mRNA

1. Assemble the following in a microcentrifuge tube, keeping the reagents on ice.

mRNA	1.5µg
Random Primers (3µg/µl)	1µl
Nuclease-Free Water to a volume of	20µl

2. Incubate the solution at 70°C for 10 minutes, then place on ice.
3. Prepare the extension mix as follows:

Component

ChipShot™ RT 5X Buffer	8.0µl
MgCl ₂ (25mM)	4.8µl
aminoallyl-dNTP mix	4.0µl
ChipShot™ Reverse Transcriptase	3.2µl
Nuclease-Free Water to a final volume of	20µl

4. Add the entire 20µl labeling mix to each RNA/primer solution, vortex, spin briefly, and incubate at 22–25°C for 10 minutes.
5. Incubate at 42°C for 2 hours, protected from light. Proceed to RNase treatment.

RNase Treatment

Add 1.0µl RNase H and 0.35µl RNase Solution to each cDNA synthesis reaction and mix gently. Incubate at 37°C for 15 minutes.

See additional protocol information in Technical Manual #TM287 available online at: www.promega.com

ORDERING/TECHNICAL INFORMATION:

www.promega.com • Phone 608-274-4330 or 800-356-9526 • Fax 608-277-2601

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For Labeled cDNA Synthesis from Total RNA or mRNA



Incubate at 70°C for 10 minutes.



Anneal primers and RNA.



Add extension mix.

Incubate at 25°C for 10 minutes.



Incubate at 42°C for 2 hours.



Add RNases. Incubate at 37°C for 15 minutes.

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INSTRUCTIONS FOR USE OF PRODUCT Z4000

Quick
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Purification of Aminoallyl cDNA

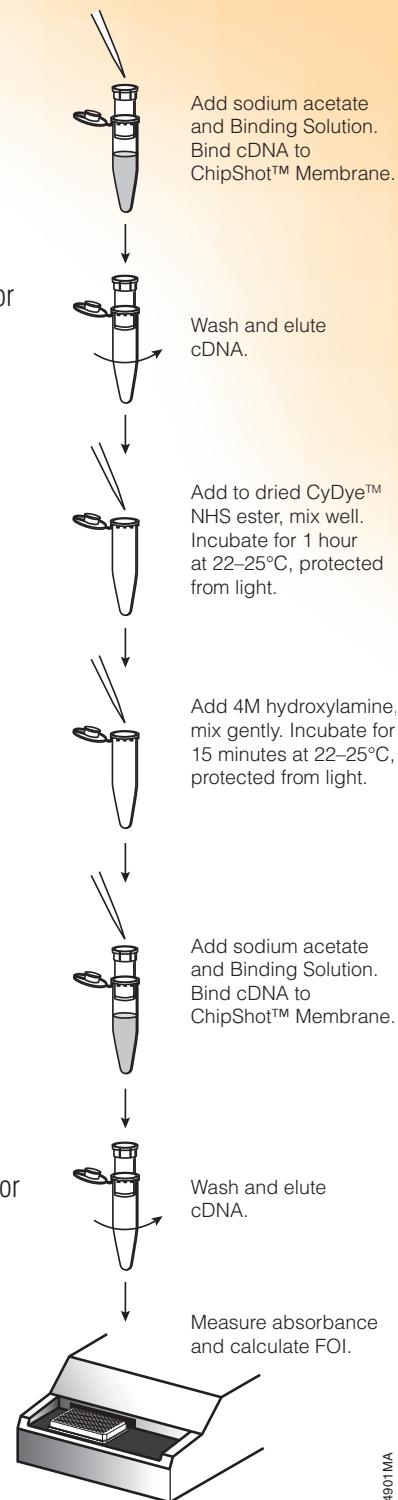
1. To 40µl of the aminoallyl cDNA, add the following components in the order listed: 4µl sodium acetate, 3M (pH 5.2), 225µl Binding Solution.
2. Vortex gently for 5–10 seconds to mix.
3. Place a ChipShot™ Membrane Column into a Collection Tube. Apply solution to the Column, cap the tube and let stand at room temperature for 5 minutes. Centrifuge at $10,000 \times g$ for 1 minute. Discard column flowthrough.
4. Wash column with 500µl of 80% ethanol, cap the tube, and centrifuge at $10,000 \times g$ for 1 minute. Discard the flowthrough.
5. Repeat Step 4 two times for a total of 3 washes.
6. Centrifuge column at $10,000 \times g$ for 1 minute to remove any ethanol.
7. Place column in a clean Collection Tube.
8. To elute purified cDNA, add 65µl of 100mM sodium bicarbonate (pH 9.0). Let the column stand for 1 minute, then centrifuge at $10,000 \times g$ for 1 minute. Proceed immediately to the dye conjugation reaction.

Conjugation of CyDye™ NHS Ester to Aminoallyl cDNA

1. Add 60µl of eluted aminoallyl cDNA to one dried aliquot of CyDye™ NHS ester.
2. Vortex gently 5–10 seconds to ensure that the CyDye™ NHS Ester is thoroughly resuspended, and incubate at room temperature (22–25°C) for 1 hour protected from light.
3. Add 20µl of 4M hydroxylamine, and vortex gently to mix. Incubate at room temperature (22–25°C) for 15 minutes, protected from light. Proceed immediately to final purification.

Purification of CyDye™-Labeled cDNA

1. To the 80µl of labeled cDNA, add the following components in the order listed: 8µl sodium acetate, 3M (pH 5.2), 440µl Binding Solution. Vortex gently to mix.
2. Place a ChipShot™ Membrane Column into a Collection Tube. Apply solution to the Column, cap the tube and let stand at room temperature for 5 minutes. Centrifuge at $10,000 \times g$ for 1 minute. Discard column flowthrough.
3. Wash column with 500µl of 80% ethanol, cap the tube and centrifuge at $10,000 \times g$ for 1 minute. Discard the flowthrough.
4. Repeat Step 3 two times for a total of 3 washes.
5. Centrifuge column at $10,000 \times g$ for 1 minute to remove any ethanol.
6. Place column in a clean Collection Tube.
7. To elute labeled cDNA, add 60µl of Elution Buffer. Let column stand for 1 minute, then centrifuge at $10,000 \times g$ for 1 minute. Eluted cDNA can be stored in a light-proof container at 4°C for several weeks.
8. Quantitate absorbance at 260, 550 and 650nm, and calculate the frequency of incorporation (FOI).



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