

ChipShot™ Direct Labeling and Clean-Up System

INSTRUCTIONS FOR USE OF PRODUCT Z4100

Quick
PROTOCOL

Labeled 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 labeling mix. Perform Cy®3 and Cy®5 reactions in separate tubes.

Component	Cy®3	Cy®5
ChipShot™ RT 5X Buffer	8µl	8µl
MgCl ₂ (25mM)	4.8µl	4.8µl
dNTP mix, total RNA	2µl	3µl
Cy®3 dCTP (1mM)	1µl	—
Cy®5 dCTP (1mM)	—	1µl
ChipShot™ Reverse Transcriptase	3.2µl	3.2µl
Nuclease-Free Water	1µl	—
final volume	20µl	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. 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.

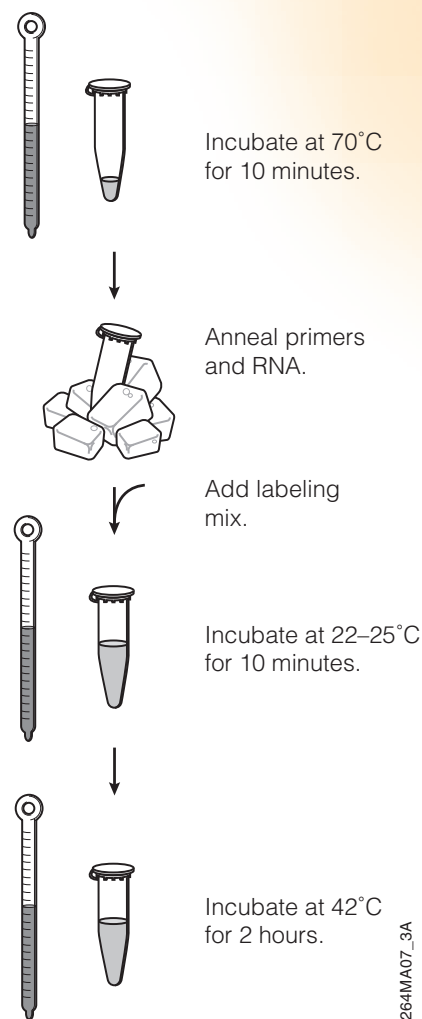
mRNA	1.5µg
Random Primers (3µg/µl)	1µl
Nuclease-Free Water to a volume of	20µl

2. Incubate the solution at 70°C for 10 minutes, then place on ice.
3. Prepare the labeling mix. Perform Cy®3 and Cy®5 reactions in separate tubes.

Component	Cy®3	Cy®5
ChipShot™ RT 5X Buffer	8µl	8µl
MgCl ₂ (25mM)	4.8µl	4.8µl
dNTP mix, mRNA	2µl	3µl
Cy®3 dCTP (1mM)	1µl	—
Cy®5 dCTP (1mM)	—	1µl
ChipShot™ Reverse Transcriptase	3.2µl	3.2µl
Nuclease-Free Water	1µl	—
final volume	20µl	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. Incubate at 42°C for 2 hours, protected from light. Proceed to RNase treatment.

For Labeled cDNA Synthesis from Total RNA or mRNA



Note: Always protect Cy®3- and Cy®5-containing solutions (including labeled products) from light.

4264MAA07_3A

ORDERING/TECHNICAL INFORMATION:

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

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RNase Treatment

1. Add 1.0µl RNase H and 0.35µl RNase Solution per cDNA-synthesis reaction and mix gently.
2. Incubate at 37°C for 15 minutes, protected from light.

Purifying Cy®-Labeled cDNA

3. To 40µl of the labeled cDNA, add the following components **in the order listed**:

Sodium Acetate, 3M (pH 5.2)	4µl
Binding Solution	225µl

4. Vortex gently for 5–10 seconds to mix.
5. 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 × *g* for 1 minute. Discard column flowthrough.
6. Wash column with 500µl of 80% ethanol, cap the tube and centrifuge at 10,000 × *g* for 1 minute. Discard the flowthrough.
7. Repeat Step 6 twice (3 washes total).
8. Centrifuge column at 10,000 × *g* for 1 minute to remove any ethanol.
9. Place column in a clean Collection Tube.
10. To elute labeled cDNA, add 60µl of Elution Buffer and let the column stand for 1 minute. Centrifuge at 10,000 × *g* for 1 minute and discard column. The eluted cDNA can be stored in a light-proof container at 4°C for several weeks.
11. Quantitate absorbance at 260, 550, and 650nm, and calculate the frequency of incorporation (FOI).

$$FOI = \frac{\text{pmol of dye incorporated} \times 324.5}{\text{ng of cDNA}}$$

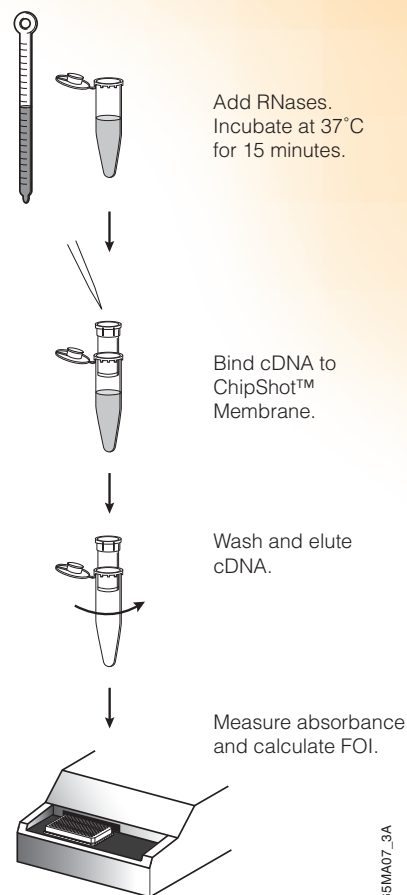
$$\text{Amount of labeled cDNA (ng)} = A_{260} \times 37 \times \text{total volume } (\mu\text{l})$$

$$\text{For Cy}^{\textcircled{3}}: \text{ pmol dye incorporated} = \frac{A_{550} \times \text{total volume } (\mu\text{l})}{0.15}$$

$$\text{For Cy}^{\textcircled{5}}: \text{ pmol dye incorporated} = \frac{A_{650} \times \text{total volume } (\mu\text{l})}{0.25}$$

These calculations were generated using the following constants: Avg. Molar Mass of dNTP = 324.5; one A_{260} unit of ssDNA = 37µg/ml; extinction coefficient of Cy³ = 150,000M⁻¹cm⁻¹ at 550nm; extinction coefficient of Cy⁵ = 250,000M⁻¹cm⁻¹ at 650nm.

See additional protocol information in Technical Manual #TM286 available online at: www.promega.com



Note: Always protect Cy³- and Cy⁵-labeled products from light.

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