



Product Information

SpyLine[™] Poly A⁺ Capture Kit

Product Codes **SPY1** and **SPY4**

Storage Temperature 2-8 °C

TECHNICAL BULLETIN

Product Description

The SpyLine[™] Poly A⁺ Capture Kit offers a simple, rapid, and cost-effective method for isolating poly A⁺ mRNA from cultured mammalian cells for direct RT-PCR. This kit features the SpyLine Poly A⁺ Plate, a 96-well PCR plate coated with oligo(dT) for selective capture of poly A⁺ mRNA from crude mammalian cell lysates.

The SpyLine Poly A⁺ Capture Kit procedure is optimized for high-throughput mRNA preparation from 96-well mammalian cell cultures containing 1,000 to 50,000 cells per well. Cells are lysed directly in the

culture plate for 5-10 minutes using the supplied lysis reagent. The crude lysate is then simply transferred to the SpyLine Poly A⁺ Capture Plate and hybridized for 30-90 minutes at room temperature. After washing, RT-PCR reagents are added directly to the SpyLine Poly A⁺ Capture Plate for subsequent RT-PCR cycling and analysis.

The captured mRNA can be used in RT-PCR or in real time quantitative RT-PCR. The SpyLine Poly A⁺ Capture Plate is compatible with most thermocyclers, including the ABI 9700 & 7700, Bio-Rad iCycler[®], and Stratagene Mx3000P.

Reagents and Techware Provided	Product Code	SPY1	SPY4
SpyLine Poly A ⁺ Capture Plate	P 3993	1 plate	4 plates
Lysis Solution	L 7417	10 mL	40 mL
Wash Solution	W 4640	40 mL	120 mL
Elution Solution	E 8024	10 mL	50 mL
Optically Clear PCR Caps	C 3616	1 pack	4 packs
AlumaSeal II [™] Film for multiwell plates	A 2350	1 pc	4 pcs

Equipment and Reagents Required But Not Provided

- 2-Mercaptoethanol (2-ME) (Product Code M 3148)
- Dulbecco's Phosphate Buffered Saline (PBS), pH 7.4 for optional cell wash (Product Code D 8662)
- RNase-free tubes (Optional)
- RT-PCR Reagents
- Filter Plate for suspension cell protocol (Product Code Z38,227-2)
- Nunc Microwell[®] Plates, 96 well Polypropylene for suspension cell protocol (Product Code P 6866)

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

RNases are ubiquitous and very stable proteins, which is a concern for any researcher attempting to isolate mRNA. The working Lysis Solution contains 2-mercaptoethanol and other RNase inhibitors, all of which inactivate RNases present in the starting cells. Conditions during lysis and washing remove RNases. Care must be taken not to introduce RNase, especially if performing the optional elution. Use RNase-free pipette tips, preferably those having an aerosol barrier. Wear latex or vinyl gloves and change them frequently. Keep bottles and tubes closed when not adding or removing their contents. The references given at the end of this bulletin are good sources of additional information on working with RNA.

Storage/Stability

Store at 2-8 °C.

Preparation Instructions

1. For best results with attached cells in a 96-well plate, grow cells in a flask or other suitable culture vessel and use before they reach maximum density or confluency. Release cells and seed 1,000-50,000 cells/well in a 96-well cell culture plate. Incubate the plate until cells are attached. The cells can then be treated as desired before isolating mRNA according to the procedure below. Allowing cells to grow in the plate will exacerbate any differences in the number of cells seeded per well and may produce unacceptable well-to-well variation.
2. Allow kit components to warm to room temperature before use.
3. Thoroughly mix reagents. Examine reagents for precipitation. If any reagent forms a precipitate, warm at 55-65 °C until the precipitate dissolves. Cool to room temperature before use.
4. Prepare sufficient Lysis Solution/2-ME Mixture for one day's use. Add 2-ME to a final concentration of 1% in Lysis Solution (10 µl of 2-ME for each 1 ml of Lysis Solution). 2-ME is required to fully inactivate RNases.

Procedure

All steps are carried out at room temperature, unless otherwise noted.

Cell Lysis

A. Cell Culture, 96-Well Adherent

1. Gently aspirate cell culture media from the plate

Optional PBS Wash

Note: PBS washes are not required, but can be used with this procedure to terminate any cell treatments.

Carefully wash the cells once or twice with 100 µl of ice cold PBS (not included).

Completely remove all PBS from the wells without disturbing the cell monolayer.

2. Add 50 µl of Lysis Solution/2-ME mixture (1% 2-ME) to each well.
3. Allow the cells to lyse for 5-10 minutes at room temperature. It is not necessary to shake or mix the cells for efficient lysis.

4. Transfer the cell lysate (50 µl) into the SpyLine Poly A⁺ Capture Plate. Pipette carefully to avoid formation of bubbles, especially in the bottom of wells, or excessive foaming of the cell lysate.
5. Cover the SpyLine Poly A⁺ Capture Plate with the provided lid.
6. Proceed with **Poly A⁺ Hybridization and Washing** section.

B. Cell Culture, Suspension cells

1. Transfer suspension cells (up to 250,000 cells in a total volume of 100-200 µl) to a multiwell glass fiber filter plate, type C, such as Product Code Z38,227-2 (not provided).
2. Place filter plate on top of a 96-well collection plate, such as Product Code P 6866 (not provided).
3. Centrifuge at 600 × g for 5 minutes at 4 °C to remove cell culture media.

Optional PBS Wash

Note: PBS washes are not required, but can be used with this procedure to terminate any cell treatments.

Carefully wash the cells once or twice with 100 µl of ice cold PBS (not included).

Place filter plate on top of a 96-well collection plate and centrifuge at 600 × g for 5 minutes at 4 °C to remove PBS.

4. Place the filter plate on top of a SpyLine Poly A⁺ Capture Plate. Add 50 µl of Lysis Solution/2-ME mixture (1% 2-ME) to each well of the filter plate. Cell lysis is carried out on the filter material.
5. Allow the cells to lyse for 5-10 minutes at room temperature. It is not necessary to shake or mix the cells for efficient lysis.
6. Spin the cell lysate into the Capture Plate by centrifugation at 600 × g for 5 min at 4 °C.
7. Cover the SpyLine Poly A⁺ Capture Plate, with the provided lid.
8. Proceed with **Poly A⁺ Hybridization and Washing** section.

Poly A⁺ Hybridization and Washing

1. Incubate the lysate in the SpyLine Poly A⁺ Capture Plate for 30-90 minutes to allow the mRNA to hybridize to the plate. 30 minutes is sufficient for detection of most mRNAs. Rare mRNAs may require incubation times of up to 90 minutes for adequate detection. Keep the SpyLine Poly A⁺ Capture Plate covered with the lid for the duration of the hybridization to minimize contamination and evaporation.
2. Aspirate the lysate from the SpyLine Poly A⁺ Capture Plate.
3. Wash the attached mRNA in the wells by adding 100 μ l of Wash Solution to the wells and aspirate completely.
4. Repeat wash by adding 100 μ l of Wash Solution to the wells and aspirating completely. Ensure all wash solution is completely removed.
5. mRNA attached to the SpyLine Poly A⁺ Capture Plate can now be used in 1- or 2-step RT-PCR or real-time RT-PCR.
6. For best results, conduct RT or RT-PCR as soon as possible after purification is completed, preferably within 4 hours. Alternatively, the mRNA has been shown to be stable on the plate for 2 months if the plate is allowed to air dry in an RNase-free environment (such as in a laminar flow hood), sealed with Alumaseal II, and stored at -20°C .

Optional Elution

If desired, the mRNA may be released from the SpyLine PolyA⁺ Capture Plate for downstream uses

1. Add 50 μ l of Elution Solution to the wells of the SpyLine Poly A⁺ Capture Plate.
2. Using a piece of the Alumaseal II Film (A 2350), seal the wells of the SpyLine Poly A⁺ Capture Plate to avoid evaporation.
3. Incubate the SpyLine Poly A⁺ Capture Plate at 65°C for 5 minutes
4. Transfer the eluted mRNA solution to RNase-free tubes or plates.

RT-PCR and Storage Notes

1. Add RT or RT-PCR reagents, seal wells with Alumaseal II film (for standard RT or RT-PCR) or with Optically Clear Caps (for real time qRT-PCR or qPCR) and proceed with thermocycling. Take care not to get fingerprints or markings that may obscure fluorescence readings on the surface of the Optically Clear Caps. Wipe caps with a Kimwipe before real-time qRT-PCR if necessary.
2. For 2-step RT-PCR, the attached oligo dT may be used to prime cDNA synthesis. The advantage of using oligo dT attached to the plate is that the cDNA will extend from the oligo dT and will then be covalently attached to the plate. Multiple PCRs can then be run from the covalently attached cDNA. Wash the plate twice with 100 μ l of Wash Solution after RT and between consecutive PCRs. For best results, start with PCR for the least abundant mRNA and increase abundance with consecutive PCR reactions.

Troubleshooting Guide

Problem	Cause	Solution
Low amount of RT-PCR product or low qRT-PCR signal	Cells were insufficiently lysed	See problem "Insufficient Lysis"
	mRNA was degraded	If 1% 2-mercaptoethanol (2-ME) is not added to the Lysis Solution before the solution is added to the cells, degradation can occur. Make sure the 2-ME is added to the Lysis Solution before lysing the cells. Take care not to contaminate the Wash Solution or the Elution Solution used for Optional Elution.
	Cells washed off of plate during media/PBS removal	Use extreme care when removing the media/PBS from the cell culture to make sure cells remain on the plate.
	Primers are for a rare mRNA	If the target mRNA is rare, the incubation time for the hybridization of the Poly A ⁺ mRNA to the SpyLine Poly A ⁺ Capture Plate should be increased to 90 minutes to get maximum binding of mRNA.
	Only one wash was done	Be sure to wash the wells two times after aspirating the Lysis Solution out of the plate. Components of the Lysis Solution can be inhibitory to RT-PCR. Two washes are necessary to remove all of the Lysis Solution.
	Failed to remove Wash Solution completely	Residual Wash Solution can affect the RT-PCR in the plate. Be sure to remove as much residual Wash Solution as is possible.
Insufficient Lysis	Lysis time was too short	Allow the Lysis Solution/2-ME to lyse the cells for at least 5 minutes before transferring to the SpyLine Poly A ⁺ Capture Plate.
	Lysis Solution/2-ME was not at Room Temperature	Allow the Lysis Solution/2-ME to reach room temperature before lysing the cells.
	Failed to remove culture medium or PBS sufficiently.	Residual media or PBS may affect the efficiency of cell lysis. Be sure to aspirate as much media or PBS as is possible from the plate before adding the Lysis Solution/2-ME.

Related Products	Product Codes
SYBR [®] Green Quantitative RT-PCR Kit	QR0100
Quantitative RT-PCR ReadyMix [™]	QR0200
Enhanced Avian HS RT-PCR Kit	HSRT-20 HSRT-100
RNA Inspector Kit	INSP1
RNaseZAP [®]	R 2020

† The PCR process is covered by patents owned by Hoffman-LaRoche, Inc.

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