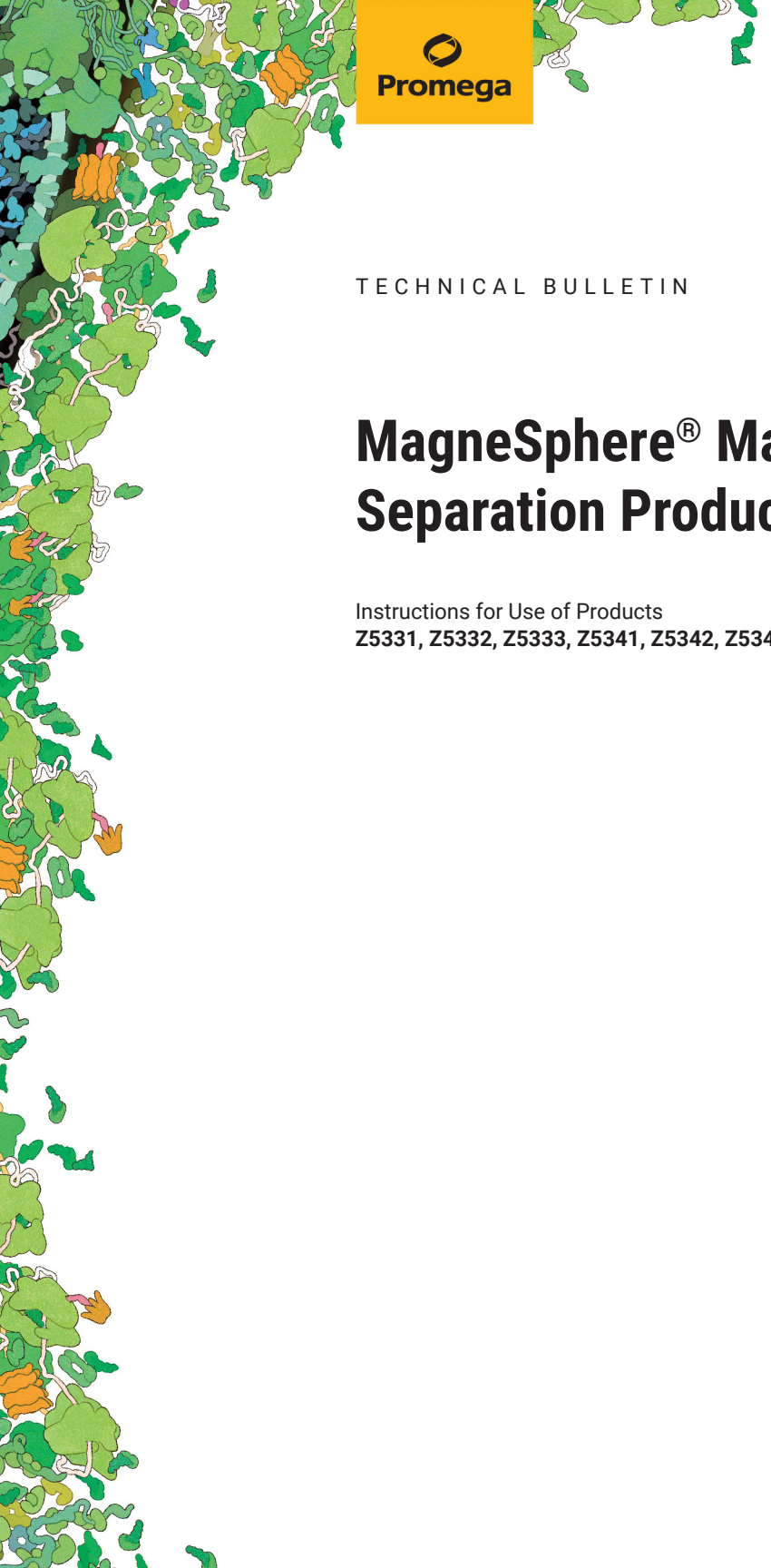

TECHNICAL BULLETIN

MagneSphere® Magnetic Separation Products

Instructions for Use of Products
Z5331, Z5332, Z5333, Z5341, Z5342, Z5343, Z5410, Z5481 and Z5482

Revised 2/25
TB246

Promega

MagneSphere® Magnetic Separation Products

All technical literature is available at: www.promega.com/protocols/
Visit the website to verify that you are using the most current version of this Technical Bulletin.
Email Promega Technical Services if you have questions on use of this system: techserv@promega.com

1. Description

Historically, bioseparations have been performed by extraction, precipitation, centrifugation, gel electrophoresis or column chromatography. The Streptavidin MagneSphere® Paramagnetic Particles (SA-PMPs) and Magnetic Separation Stands offer significant advantages over these approaches. Separations are rapid, and multiple washes or rounds of purification can be completed quickly. These products are appropriate for many magnetics-based separation protocols and are used most extensively as tools for mRNA purification.

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1. Description (continued)

The Streptavidin MagneSphere® Paramagnetic Particles consist of a magnetite core coated with streptavidin. The affinity of biotin for streptavidin ($K_d = 10^{-15}$) is one of the strongest and most stable interactions in biology. Thus, these particles combine convenient magnetic separation technology with the versatility and high affinity of the biotin-streptavidin interaction. The particles can be used with a wide variety of commercially available biotinylated reagents, including oligonucleotides, peptides, lectins, antibodies and enzymes. The biotin-streptavidin interaction cannot be reversed under nondenaturing conditions; therefore, we do not recommend the use of SA-PMPs for applications in which the biotinylated molecules need to be recovered from the SA-PMPs.

The MagneSphere® Stands and Streptavidin MagneSphere® Paramagnetic Particles are used in the PolyATtract® Systems to isolate mRNA directly from cells or tissue (PolyATtract® System 1000) or from total RNA (PolyATtract® Systems I–IV). These systems provide all of the reagents required for efficient capture of mRNA (see Section 6.D).

The MagneSphere® Magnetic Separation Stands can be used in conjunction with the SA-PMPs and any of the PolyATtract® Systems. The MagneSphere® Stands use the same samarium/cobalt magnet used in the PolyATtract® Systems.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
Streptavidin MagneSphere® Paramagnetic Particles	**(15 × 0.6ml) 9ml**	**Z5481**
	(1 × 25ml) 25ml	**Z5482**

For Laboratory Use.

PRODUCT	SIZE	CAT.#
PolyATtract® System 1000 Magnetic Separation Stand	**1 each**	**Z5410**

For use with both 15ml and 50ml tubes (includes an adapter for use with 15ml tubes).

PRODUCT	SIZE	CAT.#
MagneSphere® Technology Magnetic Separation Stand (two-position)	**0.5ml**	**Z5331**
	1.5ml	**Z5332**
	12 × 75mm	**Z5333**
MagneSphere® Technology Magnetic Separation Stand (twelve-position)	**0.5ml**	**Z5341**
	1.5ml	**Z5342**
	12 × 75mm	**Z5343**

Storage Conditions: Store the Magnetic Separation Stands and Adapter at room temperature. Store the Streptavidin MagneSphere® Paramagnetic Particles at 4°C. **Do not freeze the Streptavidin MagneSphere® Paramagnetic Particles, as this will reduce their performance.**

Important: The magnet used with this system generates a strong magnetic field. Do not place the magnet near computer screens, diskettes, pacemakers or other electronic equipment. Due to its strong attraction to metal objects, the magnet may chip upon sudden hard impact with such objects. The chips could potentially cause eye injury. Wear protective eyewear when handling this unit.

3. General Protocol for mRNA Purification from Total RNA

A schematic diagram of mRNA isolation from total RNA samples using the Streptavidin MagneSphere® Paramagnetic Particles is provided in Figure 1. The protocol described in this section is designed for use with up to 1mg of total RNA, which requires 0.6ml of SA-PMPs. The procedure may be performed conveniently using the 9ml size of the SA-PMPs (Cat.# Z5481), which is provided as 15 tubes of 0.6ml each, and the MagneSphere® Magnetic Separation Stand designed for 1.5ml microcentrifuge tubes (Cat.# Z5332). The appropriate magnetic stand for other applications can be determined by the amount of starting material (see Table 1). For other amounts of RNA, proportionally scale the amounts of SA-PMPs and Biotinylated Oligo(dT) Probe.

We do not recommend the use of long oligonucleotides for biotinylated probes, as the resulting hybrid may possess a melting temperature (T_m) that is too high to allow elution of the desired product under mild conditions.

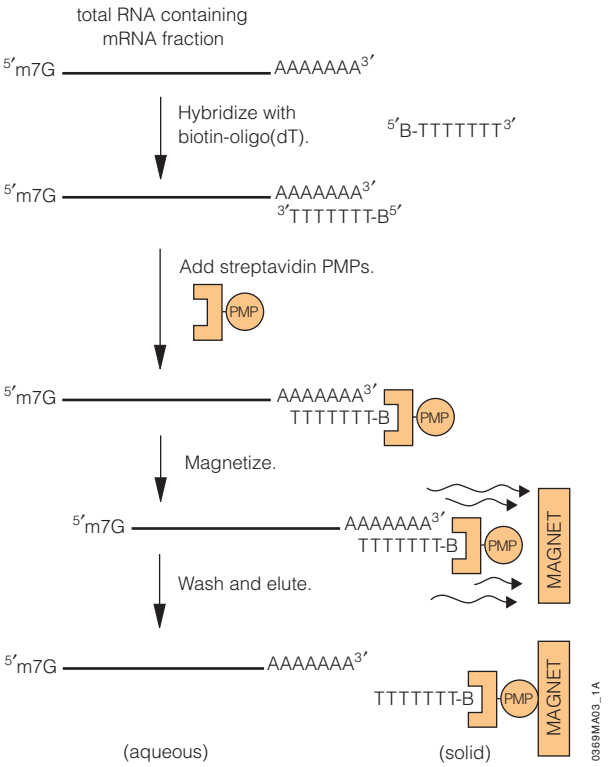

Figure 1. Schematic diagram of mRNA isolation using the SA-PMPs.

3. General Protocol for mRNA Purification from Total RNA (continued)

Table 1. Selection of MagneSphere® Magnetic Separation Stand.

Sample Size	Capture Volume Range	Tube Size	Stand (2-position)	Stand (12-position)
5–10mg tissue	180–360µl	0.5ml	Z5331	Z5341
5–35mg tissue	180µl–1.26ml	1.5ml	Z5332	Z5342
35–100mg tissue	1.26–3.6ml	12 × 75mm	Z5333	Z5343
100mg–1g tissue	3.6–36ml	15ml/50ml*	Z5410	NA
1×10^5–2.5×10^5 cells	110–275µl	0.5ml	Z5331	Z5341
2.5×10^5–1×10^6 cells	275–1,100µl	1.5ml	Z5332	Z5342
1×10^6–1×10^8 cells	1.1–18ml	15ml/50ml*	Z5410	NA

*Use sterile, polypropylene tubes. We recommend 15ml or 50ml centrifuge tubes with caps (e.g., Corning® tubes) for sample sizes ≥3.6ml. NA: not applicable.

Materials to Be Supplied by the User

(Solution compositions are provided in Section 6.C.)

- nuclease-free SSC: 20X, 0.5X and 0.1X
- water bath or heat block at 65°C
- autoclaved, RNase-free plastic tubes, 1.5ml
- sterile, RNase-free pipettes and pipette tips
- Biotinylated Oligo(dT) Probe, 50pmol/µl (Cat.# Z5261)
- Nuclease-Free Water (Cat.# P1193)

3.A. Preparation of SA-PMPs for Use

The SA-PMPs require BSA for stabilization, which is present in the storage buffer and is removed once the particles are washed. The SA-PMPs are provided at a concentration of 1mg/ml in a solution of PBS, 1mg/ml BSA and 0.02% sodium azide. The particles should be rinsed three times each with an equal volume of 0.5X SSC and used **within 30 minutes** after washing to maintain optimal performance. The particles cannot be washed and reused after the initial use.

The SA-PMPs must be completely resuspended to ensure adequate performance. Discard particles that appear to have "clumped" and cannot be dispersed. To determine if the particles are in good condition, mix by inverting the tube several times and verify that the particles remain in suspension for at least 3 minutes in a 0.5–1ml volume. If some of the particles settle out of suspension within 3 minutes, forming an easily visible pellet, they should not be used. To prevent clumping of the particles, do not freeze them or allow them to dry out.

3.B. Annealing of Probe

1. In an autoclaved, RNase-free tube, bring 0.1–1.0mg of total RNA to a final volume of 500µl in Nuclease-Free Water.

   Note: Less total RNA (50µg) may be used, but the mRNA obtained may not be detectable by spectrophotometry. Specific messages in small quantities of mRNA may be detected by RT-PCR.

2. Place the tube in a heat block at 65°C for 10 minutes.

3. Add 3µl of Biotinylated Oligo(dT) Probe and 13µl of 20X SSC to the RNA. Mix gently, and incubate at room temperature until completely cooled. This will require 10 minutes or less. While this solution is cooling, prepare stock solutions of 0.5X and 0.1X SSC.

3.C. Preparation of Stock Solution

1. Prepare 1.2ml of nuclease-free 0.5X SSC by combining 30µl of 20X SSC with 1.170ml of Nuclease-Free Water in a new, RNase-free tube.

2. Prepare 1.4ml of nuclease-free 0.1X SSC by combining 7µl of 20X SSC with 1.393ml of Nuclease-Free Water in a new, RNase-free tube.

3.D. Washing of SA-PMPs

1. Resuspend each 0.6ml tube of SA-PMPs by gently flicking the bottom of the tube until the particles are completely dispersed, and capture them by placing the tube in the magnetic stand until the SA-PMPs have collected at the side of the tube (approximately 30 seconds).

2. Carefully remove the supernatant. **Do not centrifuge the particles.**

3. Wash the SA-PMPs three times with 0.5X SSC (300µl per wash). After each wash, capture the SA-PMPs using the magnetic stand and carefully remove the wash solution.

4. Resuspend the washed SA-PMPs in 100µl of 0.5X SSC.

3.E. Capture and Washing of Annealed Oligo(dT)-mRNA Hybrids

1. Add the entire contents of the annealing reaction (Section 3.B, Step 3) to the tube containing the washed SA-PMPs.

2. Incubate at room temperature for 10 minutes. Gently mix by inversion every 1–2 minutes.

3. Capture the SA-PMPs using the magnetic stand, and carefully remove the supernatant without disturbing the SA-PMPs.

   Save the supernatant from Step 3 until you are certain that satisfactory binding and elution of mRNA has occurred.

4. Wash particles four times with 0.1X SSC (300µl per wash) by gently flicking the bottom of the tube until all particles are resuspended, then capturing the particles using the magnetic stand. After the final wash, remove as much liquid as possible without disturbing the SA-PMPs.

3.F. Elution of mRNA

1. To elute the mRNA, add 100µl of Nuclease-Free Water to the SA-PMPs and gently resuspend the particles by flicking the tube.
2. Magnetically capture the SA-PMPs, and transfer the eluted mRNA to a new RNase-free tube. Do not discard the particles.

   Note: If any particles were transferred, remove by centrifugation at 12,000 × *g* for 5–10 minutes at 4°C. Carefully transfer the RNA to a new RNase-free tube.
3. Repeat the elution by resuspending the SA-PMPs in 150µl of Nuclease-Free Water. Repeat the capture step, pooling the eluate with RNA eluted in Step 2 (250µl total volume). Save the SA-PMPs in 100µl of Nuclease-Free Water until you have verified that the mRNA has been eluted satisfactorily.

4. General Protocol for Capture of Biotinylated IgG

The following is a typical protocol to capture a biotinylated antibody using the SA-PMPs. The protocol uses biotinylated rabbit IgG (e.g., Pierce Cat.# 31826) but can be used with other biotinylated antibodies as well. The reaction requires 1mg of antibody and 10ml of SA-PMPs (at 1mg/ml), but these quantities may be scaled proportionally depending on the amount of antibody used. As a general guideline, 1mg of SA-PMPs will bind at least 70µg of biotinylated rabbit IgG.

Materials to Be Supplied by the User
(Solution compositions are provided in Section 6.C.)

- PBS, 1X
- biotinylated antibody preparation

1. Prepare the biotinylated IgG in 1X PBS. If necessary, remove any insoluble material by centrifugation at 12,000 × *g* in a microcentrifuge for a few seconds at room temperature. An antibody concentration of 4mg/ml is used in this protocol but can be varied depending on the application.
2. Resuspend SA-PMPs (1mg/ml) by gently flicking and inverting the bottle until the particles are completely dispersed. Dispense 10ml of SA-PMPs into an appropriately sized tube. Place the tube in the magnetic stand until the SA-PMPs have collected at the side of the tube (approximately 30 seconds). Carefully remove the supernatant. **Do not centrifuge the particles.**
3. Wash SA-PMPs three times, each with 10ml of 1X PBS (use a volume equal to the initial volume of SA-PMPs for each wash), capturing the particles using the magnetic stand, then carefully removing the supernatant each time.
4. Resuspend the washed SA-PMPs in 1ml of 1X PBS. The final concentration of particles is now 10mg/ml.

5. Add 1mg (250µl) of antibody preparation from Step 1 to 1ml of concentrated SA-PMPs (from Step 4). Incubate at room temperature for 30 minutes with gentle rotation of the tube.
6. After the 30-minute binding reaction is complete, magnetically capture the SA-PMPs-antibody complexes, carefully remove the supernatant and place in a clean tube.

   Note: The supernatant can be assayed for unbound IgG to determine the amount of IgG bound to the SA-PMPs. Centrifuge the supernatant at 12,000 × *g* for 5 minutes to pellet any SA-PMPs that may have been transferred. The protein concentration in the supernatant can be determined spectrophotometrically by using a protein assay or by immunoassay.
7. Wash the SA-PMPs-antibody complexes three times, each with 10ml of 1X PBS. After each wash, capture particles using the magnetic stand and carefully remove the wash solution.
8. Resuspend the SA-PMPs-antibody complexes in the appropriate buffer for your application.

5. Troubleshooting mRNA Isolation

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: **www.promega.com**. Email: **techserv@promega.com**

Problem	Causes and Comments
No mRNA eluted	No mRNA bound because salt was omitted from annealing procedure. Repeat the annealing step, adding 20X SSC to a final concentration of 0.5X.
	Insufficient cooling of annealing reaction before probe capture and wash. Add the saved supernatant back to the particles as in Section 3.E, Step 1, and continue with the procedure.
	Salt was not eliminated before elution. Wash the SA-PMPs again with Nuclease-Free Water, and check the A_{260} of this eluate.
	RNase contamination in total RNA. Evaluate the quality of total RNA by gel electrophoresis, and repeat the total RNA isolation as necessary.
RNA appears degraded on gel	RNase contamination during mRNA isolation. Repeat the entire procedure using a new total RNA sample.

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6. Appendix

6.A. Characteristics of SA-PMPs

The SA-PMPs are approximately 1.0µm in diameter and are irregularly shaped, resulting in a surface area (100–150m^2/g) that is 20–30 times greater than that of spherical particles with a similar diameter. One milligram of SA-PMPs will bind approximately 1nmol of biotinylated oligonucleotide or 100µg of biotinylated IgG.

Promega SA-PMPs are quality tested with both antibodies and nucleic acids. The SA-PMPs are free of DNase and RNase activity as determined by assays using [^{3}H]DNA and [^{3}H]RNA templates. The binding capacity is measured using biotinylated oligo(dT) and biotinylated rabbit IgG. The SA-PMPs also are tested for mRNA isolation from mouse liver using the PolyATtract® System 1000.

- **Binding Capacity:** The binding capacity of the SA-PMPs is 0.75–1.25nmol of biotinylated oligo(dT) per milligram of particles.
- **Size:** The SA-PMPs have an average diameter of 1.0 ± 0.5µm.
- **Biotin-IgG Binding Capacity:** One milligram of SA-PMPs binds at least 70µg of biotinylated IgG.
- **pH Stability:** The SA-PMPs are stable over a pH range of 5.0–9.0.
- **Temperature Stability:** The particles are stable over a temperature range of 4–65°C. Temperatures outside this range will cause the particles to clump and to be destroyed.
- **Effect of SDS:** A decrease in performance of the SA-PMPs is observed at SDS concentrations >5.0%.

6.B. Creating a Ribonuclease-Free Environment

Ribonucleases are extremely difficult to inactivate. Great care should be taken to avoid inadvertently introducing RNases into an RNA preparation during or after isolation. This is especially important if the starting material was difficult to obtain or is irreplaceable. The following notes may be helpful in preventing the accidental contamination of your sample.

1. Two of the most common sources of RNase contamination are the user's hands and bacteria or molds that may be present on airborne dust particles or laboratory glassware. To prevent contamination from these sources, sterile technique should be used when handling any reagents used for RNA isolation or analysis. Gloves should be worn at all times.
2. Whenever possible, sterile disposable plasticware should be used when handling RNA samples. These materials are generally RNase-free and do not require pretreatment to inactivate RNases.
3. Nondisposable glassware and plasticware should be treated before use to ensure that it is RNase-free. Glassware should be baked at 200°C overnight, and plasticware should be thoroughly rinsed before use with 0.1N NaOH/1mM EDTA, then with diethyl pyrocarbonate (DEPC)-treated water.

4. Autoclaving alone is not sufficient to inactivate RNases. COREX® tubes should be rendered RNase-free by treatment with DEPC and not by baking. This will reduce the failure rate of this type of tube during centrifugation. Solutions and COREX® tubes supplied by the user should be treated with 0.1% DEPC overnight at room temperature, then autoclaved for 30 minutes to remove any trace of DEPC.

   Tris buffers cannot be treated with DEPC. Use caution when weighing Tris to avoid RNase contamination, and use DEPC-treated water and glassware when preparing Tris buffers.

5. While most sources of deionized water are free of contaminating RNase activity, deionized water sources can be a potential contributor of RNase activity. If degradation of the target RNA occurs, it may be necessary to test the laboratory water source for RNase activity.

6. We recommend that chemicals used for RNA isolation and analysis be reserved solely for this purpose. Wear gloves when handling these chemicals, and use only baked spatulas and untouched weigh boats or weigh paper.

6.C. Composition of Buffers and Solutions

DEPC-treated water

Add diethyl pyrocarbonate (DEPC) to deionized water to a final concentration of 0.1% (v/v). Incubate overnight at room temperature in a fume hood. Autoclave for 20 minutes.

DEPC is a suspected carcinogen. Work in a fume hood, and follow standard laboratory safety procedures.

SSC, 20X

87.7g NaCl
44.1g trisodium citrate dihydrate

Dissolve in 400ml of DEPC-treated water. Adjust the pH to 7.2 with 10N HCl, and bring the final volume to 500ml. Dispense into aliquots. Sterilize by autoclaving.

PBS, 1X

0.2g KCl
8.0g NaCl
0.2g KH_2PO_4
1.15g Na_2HPO_4

Add components one at a time to 900ml of room-temperature deionized water, and mix until completely dissolved. Adjust the pH to 7.4 using 1N HCl or 1N NaOH, if necessary. Bring the final volume to 1 liter. If stored for long periods, filter the solution through a 0.45µm filter and store in a tightly capped bottle.

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6.D. Related Products

Systems for mRNA Purification from Total RNA

Product	Cat.#
PolyATtract® mRNA Isolation System I (Refill System for Z5200)	Z5210
PolyATtract® mRNA Isolation System II	Z5200

For Laboratory Use. Each system contains sufficient reagents for three separate mRNA isolations, each from 1–5mg total RNA. **Note:** Cat.# Z5210 **does not** contain the magnetic separation stand.

Product	Cat.#
PolyATtract® mRNA Isolation System III	Z5300
PolyATtract® mRNA Isolation System IV (Refill System for Z5300)	Z5310

For Laboratory Use. Each system contains sufficient reagents for fifteen separate mRNA isolations, each from 100–1,000µg total RNA. **Note:** Cat.# Z5310 **does not** contain the magnetic separation stand.

Systems for mRNA Isolation from Biological Samples

Product	Cat.#
PolyATtract® System 1000	Z5420
PolyATtract® System 1000 (Refill System for Z5420)	Z5400

For Laboratory Use. Each system contains sufficient reagents for mRNA isolation from up to 2g of tissue or 4×10^8 cultured cells. **Note:** Cat.# Z5400 **does not** contain the magnetic separation stand.

Systems for Total RNA Isolation

Product	Size	Cat.#
Maxwell® RSC simplyRNA Tissue	48 preps	AS1340
Maxwell® RSC simplyRNA Blood*	48 preps	AS1380
ReliaPrep™ FFPE Total RNA Miniprep System*	10 reactions	Z1001
ReliaPrep™ RNA Cells Miniprep System*	10 preps	Z6010
ReliaPrep™ RNA Tissues Miniprep System*	10 preps	Z6110
SV Total RNA Isolation System*	50 preps	Z3100

For Laboratory Use.

*Additional kit sizes are available.

Amplification-Related Products

Product	Size	Cat.#
Access RT-PCR System	100 reactions	A1250
	500 reactions	A1280

For Laboratory Use.

Product	Size	Cat.#
GoTaq® Green Master Mix	100 reactions	M7122
	1,000 reactions	M7123

For Laboratory Use. GoTaq® Green Master Mix is a premixed solution of GoTaq® DNA Polymerase, GoTaq® Green Reaction Buffer, dNTPs and Mg^{2+}. Catalog numbers may be different in Europe.

Product	Conc.	Size	Cat.#
GoTaq® DNA Polymerase	5u/µl	100u	M3001

For Laboratory Use. GoTaq® DNA Polymerase contains 5X Green GoTaq® Reaction Buffer and 5X Colorless GoTaq® Reaction Buffer. Both buffers provide 1.5mM $MgCl_2$ in the final 1X concentration. Available in additional sizes. Catalog numbers may be different in Europe.

Product	Conc.	Size	Cat.#
GoTaq® Flexi DNA Polymerase	5u/µl	100u	M8291

For Laboratory Use. GoTaq® Flexi DNA Polymerase contains 5X Green GoTaq® Flexi Buffer, 5X Colorless GoTaq® Flexi Buffer and Magnesium Chloride Solution, 25mM. Reaction buffers are magnesium-free. Available in additional sizes.
Catalog numbers may be different in Europe.

Product	Size	Cat.#
ImProm-II™ Reverse Transcription System*	100 reactions	A3800
ImProm-II™ Reverse Transcriptase*	10 reactions	A3801
	100 reactions	A3802
	500 reactions	A3803
M-MLV Reverse Transcriptase*	10,000u	M1701
M-MLV Reverse Transcriptase, RNase H Minus**	10,000u	M5301
M-MLV Reverse Transcriptase, RNase H Minus, Point Mutant**	10,000u	M3682
AMV Reverse Transcriptase*	300 units	M5101
Recombinant RNasin® Ribonuclease Inhibitor*	2,500 units	N2511

*For Laboratory Use.

**Not available for purchase in the United States.

6.D. Related Products (continued)

Other Related Products

Product	Size	Cat.#
MagnaBot® 96 Magnetic Separation Device	96-well plate	V8151
Biotinylated Oligo(dT) Probe (50pmol/µl)	35µl	Z5261
RNA Markers, 0.28–6.58kb	50µl	G3191

For Laboratory Use.

7. Summary of Changes

The following changes were made to the 2/25 revision of this document:

1. A cautionary note concerning the magnet(s) was added to Section 2.
2. Fonts and cover image were updated.
3. Related Products and trademarks were updated.