Technical Manual

PolyATtract® mRNA Isolation Systems

INSTRUCTIONS FOR USE OF PRODUCTS Z5200, Z5210, Z5300 AND Z5310.

IMPORTANT NOTICE

The Magnet Pack used with this system generates a very strong magnetic field. Do not place the Magnet Pack near computer screens, diskettes, pacemakers or other electronic equipment.

Note: Due to its strong attraction to metal objects, the Magnet Pack may chip upon sudden hard impact with such objects. The chips could potentially cause eye injury. Wear protective eyewear when handling this unit.

If you have additional questions, please contact Promega Technical Services by phone at 800-356-9526 or by e-mail: techserv@promega.com.

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PolyATtract® mRNA Isolation Systems

All technical literature is available on the Internet at: www.promega.com/tbs/ Please visit the web site to verify that you are using the most current version of this Technical Manual. Please contact Promega Technical Services if you have questions on use of this system. E-mail: techserv@promega.com

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I. Description

The PolyATtract® mRNA Isolation Systems utilize the MagneSphere® technology to eliminate the need for oligo(dT) cellulose and its associated problems. With total RNA as the starting material, the poly(A) mRNA fraction can be isolated free of other nucleic acid contamination in approximately 45 minutes. The isolated mRNA is suitable for all molecular biology applications, including in vitro translation and cDNA synthesis.

The systems use a biotinylated oligo(dT) primer to hybridize at high efficiency in solution to the 3´ poly(A) region present in most mature eukaryotic mRNA species. The hybrids are captured and washed at high stringency using streptavidin coupled to paramagnetic particles and a magnetic separation stand (Figure 1). The mRNA is eluted from the solid phase by the simple addition of ribonuclease-free deionized water. This procedure yields an essentially pure fraction of mature mRNA after only a single round of magnetic separation.

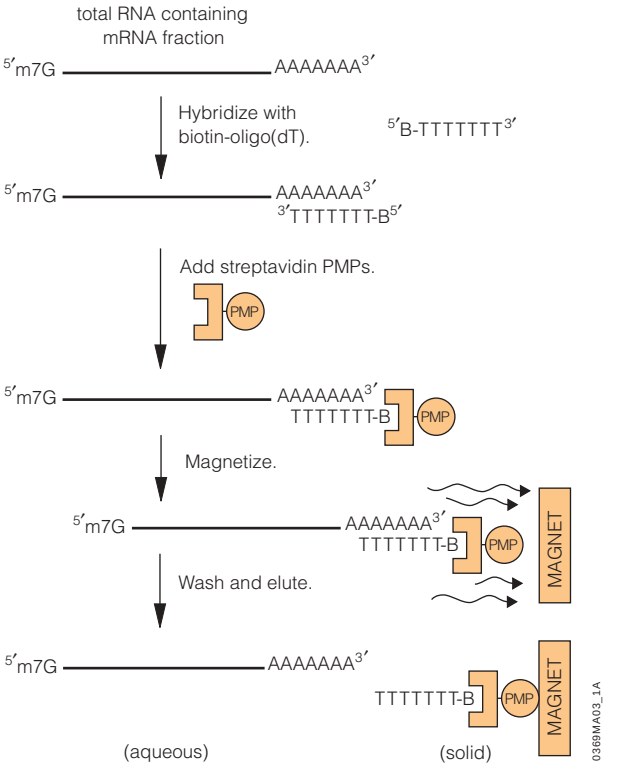

Figure 1. Schematic diagram of the PolyATtract® mRNA isolation procedure.

All of the components in the system are guaranteed to be free of contaminating ribonucleases **when used as directed** and have been thoroughly tested to ensure optimal performance. When used in combination with the SV Total RNA Isolation System, a pure fraction of intact, full-length mRNA can be isolated from a tissue or cell source in as little as 2 hours.

The PolyATtract® mRNA Isolation Systems are available in two basic configurations, the selection depending on the amount of input RNA. PolyATtract® Systems I and II are designed for larger amounts of input RNA; either system provides reagents sufficient for three isolations, each from 1–5mg of total RNA. PolyATtract® System I does not include a magnetic separation stand; PolyATtract® System II includes the stand.

PolyATtract® Systems III and IV provide the MagneSphere® Streptavidin Paramagnetic Particles in smaller aliquots, suitable for use with smaller amounts of input RNA. Each of these systems contains reagents sufficient to perform fifteen mRNA isolations, starting with 1mg or less total RNA per isolation.

II. Product Components and Storage Conditions

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

For Laboratory Use. Each system contains all the reagents and RNase-free tubes required (excluding the Magnetic Separation Stand) to perform three separate mRNA isolations, each from 1–5mg of total RNA. See Cat.# Z5200.

Product	Cat.#
PolyATtract® mRNA Isolation System II	Z5200

For Laboratory Use. Each system contains all the reagents and RNase-free tubes required to perform three separate mRNA isolations, each from 1–5mg of total RNA. Includes:

- 35µl Biotinylated Oligo(dT) Probe (50pmol/µl)
- 4.2ml 20X SSC Solution (3 × 1.4ml)
- 9ml Streptavidin MagneSphere® Paramagnetic Particles (3 × 3ml)
- 75ml Nuclease-Free Water (3 × 25ml)
- 3 each mRNA User Tubes
- 1 each MagneSphere® Magnetic Separation Stand for 12 × 75mm Tubes
- 1 Protocol

Product	Cat.#
PolyATtract® mRNA Isolation System III	Z5300

For Laboratory Use. Each system contains all the reagents required to perform fifteen separate mRNA isolations, each from up to 1mg of total RNA. Includes:

- 50µl Biotinylated Oligo(dT) Probe (50pmol/µl)
- 2.8ml 20X SSC Solution (2 × 1.4ml)
- 9ml Streptavidin MagneSphere® Paramagnetic Particles (15 × 0.6ml)
- 50ml Nuclease-Free Water (2 × 25ml)
- 1 each MagneSphere® Magnetic Separation Stand for 1.5ml Microcentrifuge Tubes
- 1 Protocol

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

For Laboratory Use. Each system contains all the reagents required (excluding the Magnetic Separation Stand) to perform fifteen separate mRNA isolations, each from up to 1mg of total RNA. See Cat.# Z5300.

Storage and Stability: If handled and stored properly, the performance of this product is guaranteed for at least 6 months from the date of purchase unless otherwise stated on the label. Store at 4°C.

Do not freeze the Streptavidin MagneSphere® Particles, as this will reduce their performance.

III. Creating a Ribonuclease-Free Environment

The following notes will help you prevent the accidental contamination of samples with ribonuclease (RNase), allowing the isolation of full-length mRNA.

1. Two of the most common sources of RNase contamination are the user's hands and bacteria and molds that may be present on airborne dust particles. To prevent this type of contamination, proper microbiological sterile technique should be observed when handling the reagents supplied with the system. **The reagents provided will be used repeatedly, so particular care must be taken to prevent contamination when opening and closing reagent tubes.** Gloves should be worn at all times.

2. Whenever possible, sterile disposable plasticware should be used for the handling of RNA. These materials are generally RNase-free and thus do not require pretreatment to inactivate RNase.

3. Nondisposable glass 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, followed by RNase-free water. COREX® tubes should be rendered RNase-free by treatment with diethyl pyrocarbonate (see below) and not by baking. This will reduce the chance of tube failure during centrifugation.

4. Solutions supplied by the user should be treated with 0.05% diethyl pyrocarbonate (DEPC) overnight at room temperature, then autoclaved for 30 minutes to remove any trace of DEPC. Likewise, glassware can be rinsed in 0.05% DEPC overnight, then autoclaved to remove residual DEPC.

Tris solutions cannot be treated with DEPC.

Note: Many sources of distilled water are free of contaminating RNase activity. Test your water source for the presence of contaminating RNase.

IV. Protocol for Large-Scale mRNA Isolation: PolyATtract® Systems I and II

This procedure is designed for use with 1–5mg of total RNA.

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

- 65°C water bath or heating block
- sterile, RNase-free plastic tubes
- sterile, RNase-free pipets and pipet tips

IV.A. Annealing of Probe

1. In a sterile, RNase-free 3ml tube, combine 1–5mg of total RNA and RNase-Free Water to a final volume of 2.43ml.
2. Place the tube in a 65°C heating block for 10 minutes.
3. Add 10µl of the Biotinylated-Oligo(dT) Probe and 60µl of 20X SSC to the RNA. Mix gently and incubate at room temperature until completely cooled. This may require up to 30 minutes, depending on the size of the tube. While this solution is cooling, prepare stock solutions of 0.5X and 0.1X SSC.

IV.B. Stock Solution Preparation

1. Prepare 5ml of sterile 0.5X SSC by combining 0.125ml of 20X SSC with 4.875ml of RNase-Free Water in a sterile, RNase-free tube.
2. Prepare 10ml of sterile 0.1X SSC by combining 50µl of 20X SSC with 9.95ml of RNase-Free Water in a sterile, RNase-free tube.

IV.C. Washing of Streptavidin-Paramagnetic Particles

The SA-PMPs require bovine serum albumin (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 in order 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.

1. Resuspend one tube (3ml volume) of the Streptavidin-Paramagnetic Particles (SA-PMPs) per isolation by gently flicking the bottom of the tube until they are completely dispersed, then 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 (1.5ml per wash), each time capturing them using the magnetic stand and carefully removing the supernatant.
4. Resuspend the washed SA-PMPs in 0.5ml of 0.5X SSC.

IV.D. Capture and Washing of Annealed Oligo(dT)-mRNA Hybrids

1. Add the entire contents of the annealing reaction (Section IV.A, Step 3) to the tube containing the washed SA-PMPs.
2. Incubate at room temperature for 10 minutes. Gently mix by inverting every 1–2 minutes.
3. Capture the SA-PMPs using the magnetic stand and carefully remove the supernatant without disturbing the SA-PMP pellet.

   Note: Save the supernatant from Step 3 until you are certain that satisfactory binding and elution of mRNA has occurred.
4. Wash the particles four times with 0.1X SSC (1.5ml per wash) by gently flicking the bottom of the tube until all of the particles are resuspended. After the final wash, remove as much of the supernatant as possible without disturbing the SA-PMP particles.

IV.E. Elution of mRNA

1. Resuspend the final SA-PMP pellet (Section IV.D, Step 4) in 1.0ml of the RNase-Free Water and gently resuspend the particles by flicking the tube.
2. Magnetically capture the SA-PMPs and transfer the eluted mRNA to one of the 2ml User Tubes provided. Do not discard the particles.

   Note: If particles have been transferred with the eluted mRNA, remove by centrifuging at 12,000 × *g* for 1 minute. Carefully transfer the RNA to a new RNase-free tube.

Refer to Section VI for recommended procedures for analysis and handling of the purified mRNA.

V. Protocol for Small-Scale mRNA Isolation: PolyATtract® Systems III and IV

This procedure is designed for use with up to 1mg of total RNA.

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

- 65°C water bath or heating block
- sterile, RNase-free plastic tubes, 1.5ml
- sterile, RNase-free pipets and pipet tips

V.A. Annealing of Probe

1. In a sterile, RNase-free 1.5ml tube, bring 0.1–1.0mg of total RNA to a final volume of 500µl in RNase-Free Water.

   Note: Less total RNA (50µg) may be used, but the mRNA obtained may not be detectable by spectrophotometry (Section VI).

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

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

V.B. Stock Solution Preparation

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

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

V.C. Washing of Streptavidin-Paramagnetic Particles

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 in order 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.

1. Resuspend one tube (0.6ml volume) of the Streptavidin-Paramagnetic Particles (SA-PMPs) per isolation by gently flicking the bottom of the tube until they are completely dispersed, then 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), each time capturing them using the magnetic stand and carefully removing the supernatant.
4. Resuspend the washed SA-PMPs in 100µl of 0.5X SSC.

V.D. Capture and Washing of Annealed Oligo(dT)-mRNA Hybrids

1. Add the entire contents of the annealing reaction (Section V.A., Step 3) to the tube containing the washed SA-PMPs.
2. Incubate at room temperature for 10 minutes. Gently mix by inverting every 1–2 minutes.
3. Capture the SA-PMPs using the magnetic stand and carefully remove the supernatant without disturbing the SA-PMP pellet.

   Note: Save the supernatant from Step 3 until you are certain that satisfactory binding and elution of mRNA has occurred.
4. Wash the particles four times with 0.1X SSC (300µl per wash) by gently flicking the bottom of the tube until all of the particles are resuspended. After the final wash, remove as much of the supernatant as possible without disturbing the SA-PMP particles.

V.E. Elution of mRNA

1. Resuspend the final SA-PMP pellet (Section V.D, Step 4) in 100µl of the RNase-Free Water and gently resuspend the particles by flicking the tube.
2. Magnetically capture the SA-PMPs and transfer the eluted mRNA to a sterile, RNase-free tube. Do not discard the particles.
3. Repeat the elution step by resuspending the SA-PMP pellet in 150µl of RNase-Free Water. Repeat the capture step, pooling the eluate with the RNA eluted in Section V.E., Step 2 (250µl total volume).

   Note: If particles have been transferred with the mRNA, remove by centrifuging at 12,000 × *g* for 1 minute. Carefully transfer the RNA to a new RNase-free tube.

Refer to Section VI for recommended procedures for analysis and handling of the purified mRNA.

VI. Analysis and Handling of Purified mRNA

VI.A. Determination of mRNA Concentration and Purity

The concentration and purity of the eluted mRNA can be determined by spectrophotometry. Pure mRNA will have an A_{260}/A_{280} absorbance ratio of ≥2.0. To estimate the mRNA concentration, assume that a 40µg/ml mRNA solution will have an absorbance of 1 at 260nm. Typically, 1–5% of total RNA is mRNA (1).

Semi-micro or micro cell cuvettes should be used for sample volumes of 1ml or less. Micro Short Cells (25mm tall) can be used to measure the absorbance of 300–400µl samples, and 50–300µl sample absorbances can be measured in 96-well UV spectrophotometers using UV translucent plates and pathlength correction values. The minimum volume that can be measured in the cuvette depends upon the position of the light beam in the instrument. Refer to the owner's manual or contact the manufacturer. Shorter pathlength cuvettes can be used to measure smaller volumes, but according to Beer's Law (ε × pathlength × concentration), a shorter pathlength requires a higher concentration of mRNA to give meaningful absorbance values. The standard pathlength of a cuvette is 1cm. Sub-micro cells are available for measuring sample volumes as small as 10µl. The compatibility of these cells with your spectrophotometer needs to be determined. Black masked cuvettes are preferable to clear wall microcuvettes for a lower signal:noise ratio. The window material of the cuvette should be polished quartz with a usable wavelength of <200nm (e.g., Starna® Spectrosil Far UV Quartz). Polystyrene and acrylic cuvettes are not suitable for measurement in the 230–280nm wavelength range unless specified.

Note: Be certain that cuvettes are RNase-free so that samples can be recovered after spectrophotometry. This can be done by washing the cuvettes briefly in 50mM NaOH followed by rinsing with sterile water.

The quality of the isolated mRNA may also be checked by denaturing agarose gel electrophoresis (1), but this may require the entire sample to be loaded when <1mg of total RNA is used for mRNA isolation. These gels can be saved and used for Northern blots. Figure 2, Panel A, illustrates the appearance of the mRNA fraction isolated from total mouse liver RNA using the PolyATtract® System. The mRNA should appear as a smear extending from approximately 8.0kb to approximately 0.5kb (depending on the tissue). The bulk of the mRNAs should be clustered around 2.0kb.

We normally see very little ribosomal contamination after the first round of selection. However, the appearance of some ribosomal bands does not indicate poor performance of the system. Figure 2, Panel B, shows a Northern blot of mRNA, which contained visible amounts of both 28S and 18S ribosomal RNAs. The blot was probed with ^{32}P-labeled biotinylated oligo(dT). Little or no hybridization is seen in the total RNA lane, whereas the mRNA-selected material shows a tremendous enrichment despite the presence of some ribosomal RNAs. Therefore, a small amount of ribosomal contamination should not affect the functionality of the mRNA in most applications.

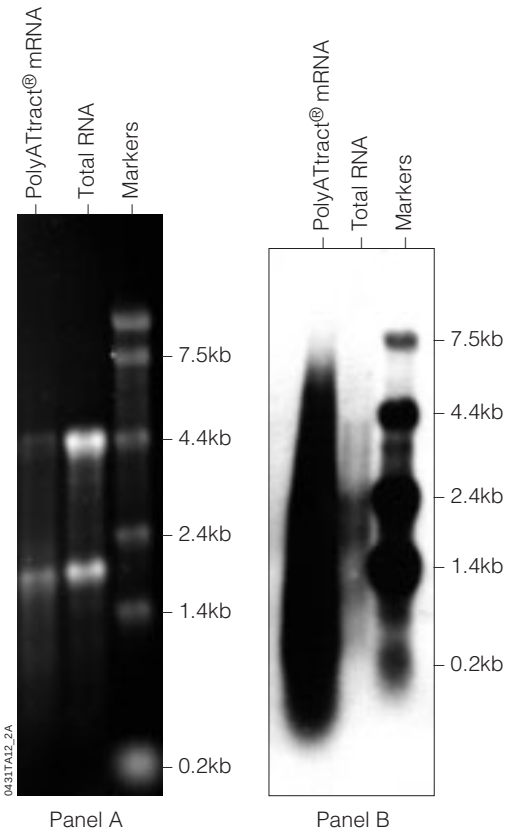

Figure 2. Northern blot of purified mRNA using the PolyATtract® mRNA Isolation System. Panel A. Ethidium bromide-stained RNA samples (5µg per lane) on a 1% denaturing agarose gel. Lane 1, mRNA fraction; lane 2, total mouse liver RNA; lane 3, 0.24–7.5kb RNA molecular weight standards. **Panel B.** RNAs in Panel A were blotted to nitrocellulose and probed with ^{32}P-labeled biotinylated oligo(dT) at 50°C in 6X SSC containing 1X Denhardt's solution and 0.1% SDS.

VI.B. Precipitation and Concentration of mRNA for Secondary Applications

While the mRNA fraction isolated with the PolyATtract® System may be sufficiently concentrated for spectrophotometric analysis, it may be too dilute for applications such as cDNA cloning and translation in vitro. If **greater than 0.5mg** of total RNA is used for mRNA isolation, the RNA may be concentrated by alcohol precipitation (2):

1. **For cDNA cloning:** Add 0.1 volume of 3M sodium acetate (pH 5.2) and 1.0 volume of isopropanol to the eluate, then incubate at –20°C overnight.

   For translation in vitro: Add 0.1 volume of 3M potassium or ammonium acetate and 1.0 volume of isopropanol to the eluate, then incubate at –20°C overnight.

2. Centrifuge at >12,000 × *g* for 10 minutes. Resuspend the RNA pellet in 1ml of 75% ethanol and centrifuge again.

3. **For short-term storage:** Dry the pellet in a vacuum desiccator for about 15 minutes, resuspend in RNase-free, deionized water at 0.5–1.0µg/µl and store at –70°C.

   For long-term storage: Store the RNA in sodium acetate/isopropanol solution at –70°C and centrifuge just prior to use.

When **less than 0.5mg** of total RNA is used as starting material, the theoretical yields of mRNA generally are expected to be low, and precipitation becomes an inefficient means of recovery. For low yield samples, freeze the mRNA eluate at –20°C for ten minutes, then dry down in a Speed Vac®. This will take approximately 2.5 hours. Samples may then be rehydrated in a nominal volume for cDNA cloning or in vitro translation. In our hands, a cDNA library can be made with mRNA isolated from as little as 100µg of total RNA.

VII. Troubleshooting

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

Symptoms	Causes and Comments
No mRNA eluted	No mRNA bound due to salt omitted from annealing step. Repeat annealing step, adding 20X SSC (to 0.5X final).
	Insufficient cooling of annealing reaction before probe capture and wash. Add the saved supernatant back to the particles as in Sections IV.D and V.D, Step 1, and continue the procedure.
	Salt not eliminated before elution. Wash final SA-PMP pellet again with deionized water and check the A_{260} of this eluate.
	RNase contamination in total RNA. Evaluate quality of total RNA by gel electrophoresis and repeat total RNA isolation as necessary. Use the RNAgents® Total RNA Isolation System to obtain high-quality total RNA.
RNA appears degraded on gel	RNase contamination during mRNA isolation. Repeat entire procedure. Reread Section III.
Low yield	High stringency wash. Repeat annealing step, adding 20X SSC (to 1.0X final) and perform the final wash step with 0.2X SSC.

VIII. References

1. Sambrook, J. and Russell, D. (2001) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
2. Wallace, D.M. (1987) Precipitation of nucleic acids. *Meth. Enzymol.* **152**, 41–8.

Additional References

1. Barron, D. (1998) Technically Speaking: Streptavidin MagneSphere® Paramagnetic Particles. *Promega Notes* **66**, 17–8.
2. Marcus, L. *et al.* (1996) PolyATtract® Systems for mRNA purification. *Promega Notes* **60**, 14–8.
3. Burke, P. (1996) Technically Speaking: PolyATtract® mRNA Isolation Systems. *Promega Notes* **56**, 27–9.

IX. Appendix

IX.A. Composition of Buffers and Solutions

The performance of this system is guaranteed when used with buffers provided with the system. For users who wish to make their own buffers, **it is important that all reagents and equipment used are RNase-free (see Section III).**

20X SSC

87.7g NaCl
44.1g sodium citrate[1]

Dissolve in 400ml of Nuclease-Free Water. Adjust pH to 7.2 with HCl and bring the volume to 500ml. Dispense into aliquots. Sterilize by autoclaving.
[1]Trisodium salt dihydrate

3M sodium acetate (pH 5.2)

408.1g sodium acetate • $3H_2O$

Dissolve sodium acetate in 800ml of water. Adjust pH to 5.2 with glacial acetic acid. Adjust the volume to 1 liter with water. Sterilize by autoclaving.

IX.B. Related Products

Systems for Total RNA Isolation

Product	Size	Cat.#
SV 96 Total RNA Isolation System*	1× 96 each	Z3500
	5× 96 each	Z3505
SV Total RNA Isolation System*	10 preps	Z3101
	50 preps	Z3100
	250 preps	Z3105
Vacuum Adapters	20 each	A1331
Vac-Man® Laboratory Vacuum Manifold, 20-sample capacity	1 each	A7231
Red Blood Cell Lysis Solution (CLB)*	200ml	Z3141
RNA Lysis Buffer (RLA)*	50ml	Z3051
RNAgents® Total RNA Isolation System*	Scalable	Z5110

*For Laboratory Use.

Systems for mRNA Isolation Directly from Biological Samples

Product		Cat.#
PolyATtract® System 1000		
with Magnetic Separation Stand*	Scalable	Z5420
without Magnetic Separation Stand*	Scalable	Z5400
PolyATtract® System 1000 Magnetic Separation Stand	1 each	Z5410

*For Laboratory Use. Each system contains sufficient reagents to isolate RNA from up to 2g of tissue or 4×10^8 cultured cells.

Amplification-Related Products

Product	Size	Cat.#
Access RT-PCR System*	500 reactions	A1280
	100 reactions	A1250
Access RT-PCR Introductory System*	20 reactions	A1260
PCR Master Mix*	100 reactions	M7502
	1,000 reactions	M7505
Taq DNA Polymerase*	500u	M1665
GoTaq® DNA Polymerase*†	100u	M3001
	500u	M3005
	2,500u	M3008
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
ImProm-II™ Reverse Transcriptase*	100 reactions	A3802
	500 reactions	A3803
AMV Reverse Transcriptase*	300u	M5101
Recombinant RNasin® Ribonuclease Inhibitor*	2,500u	N2511
RNasin® Plus RNase Inhibitor*	2,500u	N2611

*For Laboratory Use.
†Catalog numbers may be different in Europe.
**This product is not available for purchase in the United States.

Other Related Products

Product	Size	Cat.#
Biotinylated Oligo(dT) Probe (50pmol/µl)	35µl	Z5261
Streptavidin MagneSphere® Paramagnetic Particles	9ml (15 × 0.6ml)	Z5481
	25ml	Z5482
RNA Markers	50µl	G3191

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