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SOLID*script* ™ Solid Phase cDNA Synthesis Kit
Instruction Manual

Product:	SOLID*script* ™ Solid Phase cDNA Synthesis Kit (25 Reactions)
Product No:	SSP5001
Procedure:	Construction of an immobilized cDNA library *(31.112)*
Kit Storage:	See individual components for proper storage. *AMV Reverse Transcriptase is extremely thermolabile and sensitive to repeated freeze-thaw cycles. Therefore, it is recommended to divide the enzyme into 5 µl aliquots and store at -80°C. Once an aliquot is removed from -80°C, it should be stored at -20°C. AMV Reverse Transcriptase is stable at least 6 weeks when stored at -20°C. (see TECHNICAL NOTE 1)*
Stability:	Performance of this kit is guaranteed for 1 year from date of purchase.

The SOLID*script* ™ Solid Phase cDNA Synthesis Kit provides a simple single tube procedure for constructing immobilized cDNA libraries directly from tissue, cells or total RNA. mRNA is isolated on biotinylated oligo $(dT)_{25}$ complexed to streptavidin-linked Magnetic Porous Glass particles (MPG® Streptavidin Complex). The mRNA-MPG® Streptavidin Complex is used without further modification in the reverse transcriptase reaction using the immobilized oligo $(dT)_{25}$ to prime the reaction. The entire protocol takes approximately two hours and is completed in a single tube, thus eliminating potential loss of template. Other benefits include rapid manipulations with no phenol extractions, ethanol precipitations, or centrifugations. The fact that the cDNA library is immobilized on a magnetic particle allows for an easy one-step removal of template after a PCR reaction.

The SOLID*script* ™ Solid Phase cDNA Synthesis Kit provides enough reagents to construct up to 25 immobilized cDNA libraries. Each immobilized cDNA library yields sufficient product to perform a minimum of 20 separate PCR reactions. Immobilized cDNA libraries are stable for at least 18 months when stored at 4°C. Immobilized cDNA libraries are ready for use in:

- PCR
- cDNA cloning
- aRNA amplification
- cDNA amplification
- RACE

Kit Components

7.5 mg	MPG® Streptavidin Biotinylated Oligo $(dT)_{25}$ Complex (10 mg/ml)	25 µl	AMV Reverse Transcriptase (20 U/µl)
		25 µl	RNase Inhibitor (20 U/µl)
26 ml	2X Tissue Extraction/Hybridization Buffer	200 µl	dNTP mix (2.5 mM each)
2 x 20 mg	Dithiothreitol	150 µg	Control Mouse Liver Total RNA
25 ml	Hybridization Wash Buffer I	8 µl	Control PCR Primers (mouse p53)
12.5 ml	Hybridization Wash Buffer II	5 ml	TE Buffer
20 ml	Pre-RT Wash Buffer	1 ml	Nuclease-free Water
250 µl	5X RT Buffer		

Materials Not Included With Kit:

Nuclease-free water for dilution of the 2X Tissue Extraction/Hybridization Buffer
Microcentrifuge tubes
Magnetic Particle Separator (Product No.: MPS0301, MPS0001)

PREPARATION OF IMMOBILIZED mRNA *(follow either Protocol A or B)*

Protocol A: Starting from Tissue or Cells

NOTES:
1. If starting from total RNA, go to Protocol B on page 3.
2. If you wish to determine the yield of mRNA, see Technical Note 6 before proceeding.

Preparation of MPG® Streptavidin Complex

- Remove all kit components, ***except AMV Reverse Transcriptase***, thaw and then place on ice until ready to use. AMV Reverse Transcriptase should remain at -80°C until ready to use (see Technical Note 1).
- Prepare 2X Tissue Extraction/Hybridization Mixture by adding the 2 tubes of Dithiothreitol to the vial containing the 2X Tissue Extraction/Hybridization Buffer, mix by inversion to dissolve. Unused portions may be stored at -20ºC. This mixture forms a precipitant during storage. Warm to redissolve prior to use.
- Prepare 1X Tissue Extraction/Hybridization Mixture by mixing 10 ml of the 2X Tissue Extraction/Hybridization Mixture with 10 ml of nuclease-free water (DEPC-treated water is recommended). Dissolve precipitants before dilution and then chill the 1X Tissue Extraction/Hybridization Mixture prior to use. 1X Tissue Extraction/Hybridization Mixture may also be stored at -20°C.

1. Vortex the MPG® Streptavidin Complex to fully suspend the particles. Transfer 30 µl (300 µg) of MPG® Streptavidin Complex to a 1.5 ml nuclease-free microcentrifuge tube. Magnetically separate using a magnetic particle separator and carefully remove the supernatant. (see Technical Note 2 for detailed instructions on magnetic separation)

2. Resuspend the MPG® Streptavidin Complex in 50 µl of 1X Tissue Extraction/Hybridization Mixture and put aside until ready for hybridization.

Capture of mRNA on MPG® Streptavidin Complex

1. *Tissue:* Isolate and weigh fresh tissue of interest. To minimize mRNA degradation, quickly place the tissue in a 50 ml tube and add enough ice cold 1X Tissue Extraction/Hybridization Mixture so that the final concentration of tissue is between 0.05-0.10 grams per ml. (200 mg of tissue is sufficient for each isolation) For isolation of mRNA from plant tissue bring the concentration to 0.1-0.2 g/ml.

   Cells: For cells grown as a monolayer, add 1 ml of ice cold 1X Tissue Extraction/Hybridization Mixture for every 10^6-10^7 cells. Detach cells using a cell scraper and transfer to a 50 ml conical tube. For cells grown in suspension, pellet cells in a 50 ml conical tube and add 1 ml 1X Tissue Extraction/Hybridization Mixture for every 10^6-10^7 cells.

2. Disrupt tissue and cells by homogenization using 30-second pulses for 1-2 minutes with a polytron. For plant tissue, grind the tissue using a mortar and pestle on a -70°C ice bath (liquid nitrogen or acetone/dry ice). Transfer 1.0-1.5 ml aliquots of homogenate to fresh microcentrifuge tubes. For additional information on homogenization techniques see Technical Note 4.

3. Centrifuge for 45 seconds to 1.5 minutes at 14,000 x g.

4. While the homogenate is spinning, magnetically separate and carefully remove the buffer from the MPG® Streptavidin Complex processed in *"Preparation of MPG® Streptavidin Complex"* Step 2.

5. When the centrifugation in Step 3 is complete, add 1.0 ml of tissue homogenate supernatant to the MPG® Streptavidin Complex. Vortex well and incubate with end-over-end mixing for 5 minutes at room temperature. Magnetically separate and carefully remove the supernatant.

6. Wash the mRNA-bound MPG® Streptavidin Complex by resuspending the mRNA-bound MPG® Streptavidin Complex in 0.5 ml of Hybridization Wash Buffer I. Magnetically separate and carefully remove the supernatant. Repeat this washing step one more time.

7. Continue with the washing procedure once with 0.5 ml of Hybridization Wash Buffer II, and three times with 0.25 ml of ice cold Pre-RT Wash Buffer. Magnetically separate and carefully remove the supernatant. (NOTE: Complete washing of the particles is essential to remove any residual detergent that may remain from the mRNA isolation steps.)

8. The mRNA-bound MPG® Streptavidin Complex is ready for immobilized cDNA library construction. Proceed immediately to "*IMMOBILIZED cDNA LIBRARY CONSTRUCTION*" on page 4.

Protocol B: Starting from Total RNA

NOTE: If you wish to determine the yield of mRNA, see Technical Note 6 before proceeding.

Preparation of MPG® Streptavidin Complex

- Remove all kit components, ***except AMV Reverse Transcriptase***, thaw and then place on ice until ready to use. AMV Reverse Transcriptase should remain at -80°C until ready to use (see Technical Note 1).
- Prepare 2X Tissue Extraction/Hybridization Mixture by adding the 2 tubes of Dithiothreitol to the vial containing 2X Tissue Extraction/Hybridization Buffer, mix by inversion to dissolve. Unused portions may be stored at -20ºC. This mixture forms a precipitant during storage. Warm to re dissolve prior to use.

1. Vortex the MPG® Streptavidin Complex to fully suspend the particles. Transfer 30 µl (300 µg) of MPG® Streptavidin Complex to a 1.5 ml nuclease-free microcentrifuge tube. Magnetically separate using a magnetic particle separator and carefully remove the supernatant. (See Technical Note 2 for detailed instructions on magnetic separation)

2. Resuspend the MPG® Streptavidin Complex in 125 µl of the 2X Tissue Extraction/Hybridization Mixture and put it aside until ready for hybridization.

Capture of mRNA on MPG® Streptavidin Complex

1. Add 75 µg of total RNA to a clean nuclease-free microcentrifuge tube. Bring the total volume to 125 µl with nuclease-free water.

2. Disrupt the secondary structure of the total RNA by heating at 65°C for 2-3 minutes.

3. Transfer the 125 µl of heat disrupted total RNA to the tube containing the MPG® Streptavidin Complex in 125 µl 2X Tissue Extraction/Hybridization Mixture (prepared in "*Preparation of MPG® Streptavidin Complex*" Section, Step 2 above). The final concentration of Tissue Extraction/Hybridization Mixture will now be 1X.

4. Vortex and incubate 1-3-minutes at room temperature with end-over-end mixing. Magnetically separate and carefully remove the supernatant.

5. Wash the mRNA-bound MPG® Streptavidin Complex by resuspending in 0.5 ml of Hybridization Wash Buffer II. Magnetically separate and carefully remove the supernatant. Repeat this washing step two more times.

6. Continue with the washing procedure, washing the mRNA-bound particles with 0.25 ml of ice cold Pre-RT Buffer three times. Magnetically separate and carefully remove the supernatant. (NOTE: Complete washing of the particles is essential to remove any residual detergent that may remain from the mRNA isolation steps.)

7. The mRNA-bound MPG® Streptavidin Complex is ready for immobilized cDNA library construction. Proceed immediately to "*IMMOBILIZED cDNA LIBRARY CONSTRUCTION*" below.

IMMOBILIZED cDNA LIBRARY CONSTRUCTION

1. Add to the mRNA-bound MPG® Streptavidin Complex:

30 µl	nuclease-free water
10 µl	5X RT Buffer
8 µl	dNTP mix
1 µl	RNase Inhibitor
1 µl	AMV Reverse Transcriptase

2. Mix well by gently vortexing, making sure the particles are well suspended.

3. Incubate at 42°C for one hour with frequent mixing (gently vortexing once every 10-15 minutes) to keep the beads suspended. Alternatively, mixing can be accomplished using an end-over-end mixer, or hybridization rotator placed in a 42°C oven.

4. Magnetically separate and carefully remove the supernatant. Resuspend the cDNA-bound particles in 50 µl of TE Buffer. Discard the supernatant and wash two more times with 50 µl of TE Buffer. (NOTE: DO NOT HEAT DENATURE OR HEAT INACTIVATES THE ENZYME. THE IMMOBILIZED cDNA-LIBRARY SHOULD NOT BE HEATED ABOVE 42°C PRIOR TO PCR.)

5. Resuspend the cDNA-bound particles in 50 µl of TE buffer and store at 4°C. The immobilized cDNA library is stable for at least 18 months when stored under these conditions.

NOTE: Control mouse total RNA and mouse p53 PCR primers are included to evaluate the performance of this kit. See Technical Note 7 for instructions on the use of these kit controls.

RECOMMENDED CONDITIONS FOR PCR

For a 50 µl PCR reaction, add the following:

2.5 µl	Immobilized cDNA-library in TE (from step 5 above) (6 µg/µl)
10 mM	Tris-HCl, pH 8.3
50 mM	KCl
200 µM	each dNTPs
0.2-1.0 µM	each primer
2.5 Units	thermostable DNA polymerase
1.5 mM	$MgCl_2$
0.2 mg/ml	nuclease-free BSA (non-acetylated)

Note: For running the kit control, use 4 µl of the kit control primer and add a $MgCl_2$ concentration of 1.5 mM.

1. The above conditions (shown as the final concentration of each component in a 50 µl PCR reaction) are suggested as a starting point for optimization of the PCR. NOTE: *If you prefer to use a pre-made PCR reaction buffer, please check the buffer components and the concentration of the components vs. the recommended components above.*

2. The optimal amount of cDNA-bound particles in the PCR reaction is 15 µg (2.5 µl of a 6 µg/µl stock) in a 50 µl reaction. Signal is generally not increased by adding a greater amount of immobilized library to the PCR reaction. Concentrations of immobilized library exceeding 30 µg per 50 µl reaction are inhibitory.

3. The optimal $MgCl_2$ concentration may need to be determined empirically for specific primers and target sequence. However, 1.5 mM $MgCl_2$ works well for most PCR reactions.

4. Addition of nuclease-free BSA (non-acetylated) in the PCR reaction greatly enhances signal in solid phase PCR reactions. The BSA concentration (0.2 mg/ml) has been optimized and should not need further adjustment.

5. Taq-*FORCE*™ Amplification System is optimized for performance of this kit. This system contains Unmodified Taq™ DNA polymerase, MIGHTY™ Buffer (10X PCR buffer) and dNTP mix (10 mM). The MIGHTY™ Buffer contained in this kit is a unique buffer which has been shown to enhance signal when used in difficult PCR reactions. This buffer alleviates the requirement for BSA in the PCR reaction.

6. Non-specific high molecular weight PCR products (*i.e.* EtBr-staining material remaining in the wells of agarose gels after electrophoresis) can be formed when Taq polymerase is added to a cold PCR reaction mix. If this problem occurs, a "hot-start" PCR should eliminate this problem. Examples of hot-start PCR include: addition of Taq polymerase after the reaction reaches 70°C; use of wax-entrapped $MgCl_2$; or, use of antibodies against Taq polymerase that release active enzyme above 70°C.

   The optimal PCR cycling parameters for the kit control are:

94°C	5 minutes	
94°C	45 seconds	X 35
60°C	45 seconds	
68°C	90 seconds	
68 °C	7 minutes	

TECHNICAL NOTES

1. This kit comes with enough reagents for the construction of 25 immobilized cDNA libraries. AMV Reverse Transcriptase is extremely thermolabile and sensitive to repeated freeze-thaw cycles. Therefore, it is recommended to divide the enzyme into 5 µl aliquots and store at -80°C. Once an aliquot is removed from -80°C, it should be stored at -20°C. However, AMV Reverse Transcriptase is stable for at least 6 weeks, with no loss in activity, when stored at -20°C.

2. Instructions for use of magnetic separator:

Separation

Insert the test tube in the magnetic separator. Let the tube remain in the magnetic separator for 30 seconds, or until all of the particles are drawn to the wall of the tube. Once the solution is clear, remove the supernatant while leaving the tube in the magnetic separator.

Washing

Remove the tube from the magnetic separator. Add the wash solution along the wall of the test tube to resuspend the particles. Vortex to ensure even suspension of the particles. Insert the tube in the magnetic separator for 30 seconds, or until all of the particles are drawn to the wall of the tube.

Resuspension

Remove the tube from the separator. Add the appropriate solution along the wall of the test tube to resuspend the particles. Vortex to ensure even suspension.

3. Keeping the MPG® Streptavidin Complex well suspended during incubations greatly improves the efficiency of mRNA hybridization. This is best accomplished by end-over-end mixing. A laboratory rotator or hybridization rotator can be used for this purpose.

4. Complete homogenization of tissue and cells is essential for maximum mRNA capture on the MPG® Streptavidin Complex. Freshly isolated tissue or cells, immediately disrupted in ice-cold homogenization buffer, is the best source for obtaining intact mRNA. The most effective method for disrupting fresh tissue and cells is to homogenize using a polytron. Alternative methods include: using a Dounce homogenizer; multiple passes through a syringe fitted with an eighteen gauge needle; grinding frozen tissue or cells in liquid nitrogen with a mortar and pestle; and sonication. Although fresh tissue is recommended, tissue or cells snap frozen in liquid nitrogen and stored at -80°C can be used.

5. The SOLID*script* ™ Solid Phase cDNA Synthesis Kit procedure is based on immobilizing a cDNA library on 300 µg of solid phase which can be used for a minimum of 20 PCR reactions. The procedure can be scaled up or down by proportionately adjusting each component volume. Increasing the concentration of solid phase in the PCR reaction is not recommended.

6. To determine the yield of mRNA isolated, use 600 µg (in place of the 300 µg) MPG® Streptavidin Complex in Step 1 of "*Preparation of MPG® Streptavidin Complex* ". Double the volumes of each component through Step 5 of Protocol A or Step 4 of Protocol B in the Section "*Capture of mRNA on MPG® Streptavidin Complex*". Resuspend the particles in 1 ml Hybridization Wash Buffer I and transfer 300 µg (500 µl) to a fresh 1.5 ml microcentrifuge tube (Tube 2). Continue through the protocol with the first tube. Wash the particles in Tube 2 twice with 500 µl Hybridization Wash Buffer II. Remove the supernatant and resuspend in 20 µl TE. Elute the mRNA by heating at 65°C for 2 minutes. Place the tube in the magnetic separator and remove the supernatant containing the eluted mRNA. This sample should contain approximately 1 µg mRNA. Yield and purity can be determined by measuring the optical density (OD) of the isolated mRNA at wavelengths of 260 nm and 280 nm.

   Yield of mRNA (µg/ml)=(OD_{260})(40) (dilution factor)
   Purity of mRNA = $(OD_{260})/(OD_{280})$
   $(OD_{260})/(OD_{280})$ of pure mRNA is $\cong$ 2.0

7. Use of Control: Control mouse liver total RNA (150 µg/250 µl) and a set of mouse p53 PCR primers are included to test the performance of this kit in the event of poor results with your experimental samples. The control mouse liver total RNA (125 µl) is substituted for the total RNA added in Step 1 of *"Capture of mRNA on MPG® Streptavidin Complex"*, "*Protocol B: Starting from Total RNA".* Following the construction of the immobilized cDNA library, the mouse p53 PCR primers are used to prime a PCR reaction. (See the recommended conditions for the PCR reaction on page 5.) The PCR reaction should generate a 371 base pair amplicon.

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