



XPRESS EZ-RNA YEAST KIT

Catalogue Numbers

EZ-900 2 X 96 Purifications

EZ-1000 6 X 96 Purifications

EZ-1100 2 X 96 Purifications, XS Buffer

EZ-1200 6 X 96 Purifications, XS Buffer

INTRODUCTION

The Xpress EZ-RNA Yeast kit is designed for the high-throughput purification of total cellular RNA from up to 1×10^7 yeast cells per well in a 96-well plate format with no phenol-chloroform extractions. Lysates are first prepared from yeast spheroplasts using a table-top centrifuge, and then RNA is purified from the lysates using a silica membrane filter plate on a vacuum manifold.

In the first step, yeast cells are treated with cell wall degrading enzymes to create spheroplasts. A lysate is then prepared from the spheroplasts which can be used directly or stored frozen for up to a month. Ethanol is added to the samples, which are then added to the filter plates. The plate is then washed to further remove protein, buffer components and other contaminants using two ethanol-containing wash buffers and the final RNA product is eluted in RNase-free water. The final RNA product is high quality total cellular RNA that can be used directly for quantitative RT-PCR and other downstream applications. A protocol to treat the final RNA product with DNase I is also included, since yeast plasmid DNA is also copurified along with the RNA in this protocol. Small RNAs (<200 nucleotides) such as tRNA are not efficiently isolated using this kit.

The Xpress EZ-RNA yeast kit 96-well silica membrane plates have a standard Society for Biomolecular Screening (SBS) footprint and are compatible with a variety of automated liquid handling workstations, all 96-well-compatible vacuum manifolds and most, if not all, 96-well plate-compatible rotors.

The preparation of lysates from spheroplasts and the RNA purification protocol will take approximately 1 hr. each to perform, however two vacuum manifolds in parallel can be used to process two 96-well plates in nearly the same amount of time. The EZ-1100 and EZ-1200 kits are the same as the EZ-900 and EZ-1000 kits, respectively, except that they contain twice the amount of wash buffers to allow for sufficient buffer for use with robotic workstations.

STORAGE CONDITIONS

All contents of the Xpress EZ-RNA yeast kit should be stored at room temperature, except for the yeast spheroplast enzyme which should be stored at -20°C . The kit is stable for one year under these conditions.

SAFETY INFORMATION

The MSDS for this kit is available online at www.expressbiotech.com.

TECHNICAL ASSISTANCE

Please refer any technical questions to techquestions@expressbio.com.

CONTENTS PER TWO-PACK KIT

Product	Catalogue	Per Kit
96-well deep well plates	EZ-501	2
Processing plates	EZ-304	2
Plate sealers	EZ-305	4
Filter plates	EZ-101	2
Collection plates	EZ-102	4
Spheroplast buffer	EZ-901	1 X 200 ml
Spheroplast enzyme	EZ-902	2 X 1 ml
Yeast binding buffer	EZ-903	1 X 40 ml
RNase-free water	EZ-104	1 X 20 ml
Wash buffer 1 concentrate	EZ-105	1 X 70 ml ^a
Wash buffer 2 concentrate	EZ-106	1 X 60 ml ^a
RNase-free porous tape	EZ-107	4
Instruction manual	NA	1

^aXS buffer kit EZ-1100 contains two bottles.

NOTES BEFORE STARTING

General Considerations

Great care should be taken to not introduce ribonucleases (RNases) into the experiment. Hands and dust are the most common sources of RNase. Therefore, disposable gloves should be worn at all times and disposable plasticware should be used. Similarly, RT-PCR reactions should be set up in a biological safety hood or PCR workstation.

Yeast DNA plasmids copurify with yeast RNA. If DNA plasmids will interfere with your analyses then use the DNase I treatment protocol.

Sample Size and RNA Yield

The Xpress EZ-RNA yeast kit can be used to isolate total cellular RNA from up to 1×10^7 yeast cells per well. The yeast may be grown as overnight cultures or as required. The total RNA yield is directly proportional to the number of cells utilized when up to 1×10^7 yeast are used, which yields approximately 4 μg RNA per well.

Buffer Concentrates

Wash Buffers 1 and 2 are provided as concentrates that require the addition of 100% ethanol to them before use.

Increasing Throughput

The protocol can be expedited considerably when the wash buffers are applied to the wells of the filter plate using a wash bottle to deliver the buffer rather than by pipetting.

Reagents and Equipment to be Supplied by the User

- Pipetteman (multichannel pipettors desirable) with sterile RNase-free tips
- Disposable gloves
- 100% ethanol
- 14.5M β -mercaptoethanol
- Laboratory-grade adhesive tape
- Paper towels
- Any 96-well plate-compatible vacuum manifold
- A rotary shaker
- A vacuum source with a capacity of 18 liters/min. The use of a weak vacuum may reduce the RNA yield and purity
- A table-top centrifuge capable of providing 2000g with rotors that can accommodate 96-well plates (including GH3.8, GH3.8A and J34.3).

XPRESS EZ-RNA YEAST KIT PROTOCOL

Before starting: The yeast binding buffer requires the addition of 20 μ l 14.5M β -mercaptoethanol (BME) per ml before use. If crystals appear in the yeast binding buffer then it should be warmed briefly at 37°C to solubilize it. Yeast spheroplast buffer requires the addition of 10 μ l/ml yeast spheroplast enzyme and 2 μ l/ml BME just before use. The wash buffer 1 concentrate requires the addition of 70 ml of 100% ethanol before it can be used, while the wash buffer 2 concentrate requires that 240 ml of 100% ethanol is added to it before use. Both of the wash buffers are stable for one year after the addition of ethanol.

A. Preparation of lysates

1. Thaw and vortex the spheroplast enzyme before use. Add 1 ml spheroplast buffer containing 10 μ l/ml spheroplast enzyme and 2 μ l/ml BME per well of the 96-well deep well plate and then add up to 100 μ l containing up to 1×10^7 cells of yeast culture to the wells. Seal the plate with a plate sealer, secure the plate to a rotary shaker using adhesive tape (not provided) and shake the plate for 30 min. at room temperature at 200 rpm.

This treatment digests the yeast cell wall to create yeast spheroplasts. If more than 100 μ l of yeast culture are required then spin down the culture (500g X 5 min.), remove the supernatants and resuspend the cells in 100 μ l PBS before beginning.

2. Spin down the spheroplasts (500 g X 5 min.) and pour off the supernatants.

Do not smash the plate onto paper towels to remove all traces of buffer from the wells. Small amounts of supernatant will not interfere with the rest of the protocol. The yeast spheroplasts may form loose pellets which can be dislodged if supernatants are removed too vigorously. These pellets should be visible when between 1×10^6 and 1×10^7 yeast are utilized.

3. Add 200 μ l of yeast binding buffer containing 20 μ l/ml BME to each well. Resuspend the spheroplasts and transfer this to the corresponding wells of the processing plate. Seal the plate using the plate sealers and centrifuge it 2000g X 5 min.

This step removes traces of unlysed yeast spheroplasts and yeast, which will otherwise clog the filter plate. A visible yeast pellet may only be apparent when large quantities of yeast ($>1 \times 10^7$) are employed. There is no need for concern if a small pellet is observed at this stage. The process will work well as long as the pellet is not disrupted when the supernatants are collected in step 4. The filter plates can accommodate up to 1×10^8 yeast to prepare high quality RNA, however yeast spheroplast formation is incomplete under these conditions and the linearity of RNA content with yeast cell number no longer holds since the capacity of the filter plate to bind RNA becomes limiting.

4. Tilt the plate slightly to face you and gently transfer 150 μ l supernatant from each well to the corresponding well of a collection plate by slowly inserting the pipette tip into the supernatant as you draw the liquid out. Avoid the pellet and the bottom of the tube.

The preparation of the lysate is now complete and the RNA in it is stable. The protocol can be stopped briefly now at room temperature (~1 hr.). Samples that will be processed later in the day should be stored at 2-8°C, while samples that will be processed much later should be stored at -80°C where they are stable for up to one month.

Purification of RNA

1. This portion of the protocol may be performed by hand or using a robotic workstation containing a vacuum manifold. Frozen plates containing yeast spheroplast lysates must be thawed to room temperature before proceeding. Place the filter plate onto the vacuum manifold. Add 150 μ l of 100% ethanol per well to the lysate samples, pipette the plate well contents up and down three times to mix it and add the contents to the corresponding well of the filter plate. Turn on the vacuum pump for 30 seconds or until all of the sample material has been drawn through the wells of the filter plate. Then release the vacuum pressure from the plate assembly before turning off the vacuum.

If some of the plate wells will not receive any samples, first cover the wells that will not be used with ordinary laboratory adhesive tape (not supplied). This helps to increase the vacuum pressure to the wells that are used, and these covered wells can be used another day.

As with all 96-well applications, some small differences in efficiency may be experienced when the outer wells of the plate, and in particular the plate corner wells, are used. The inner wells of the plate should be employed first whenever possible when experiments are designed.

2. Wash the wells by adding 700 μ l wash buffer 1 (which contains the added ethanol) per well and apply the vacuum as above.

3. Wash the wells twice by adding 700 μ l wash buffer 2 (which contains the added ethanol) and apply the vacuum as above.

4. Remove the filter plate from the vacuum manifold and pat it down firmly on a stack of paper towels until no further liquid is released. Return the filter plate to the vacuum manifold assembly and turn on the vacuum for 5 min. to completely dry the membrane.

The plate wells need to be dry in order to prevent alcohol carryover in the final elution step. Some vacuums may accomplish this in 3 min., while others may require 10 min. A 5 min. drying step is sufficient for most users.

5. Place the filter plate into the bottom half of the 96-well collection plate and add 50 μ l of RNase-free water per well. Cover the plate with the RNase-free porous tape, wait 1 min., and then centrifuge the filter plate/collection plate assembly (900g X 2 min.) to elute the final RNA product. Covering the plate helps keep dust out of the plate. Repeat the elution using 50 μ l RNase-free water as above.

The use of one 50 μ l elution rather than two elution steps will provide you with a slightly more concentrated RNA product, however the absolute yield of RNA will be reduced. The dead volume of the filter plate wells is 20 μ l, so one can expect to recover approximately 30 μ l or 80 μ l of final RNA product when one or two elution steps are used, respectively. It is best to use the RNA directly after isolation. If this is not possible then freeze the RNA to -80°C until it is needed.

6. DNase digestion (optional). Yeast plasmid DNA will also be copurified with yeast RNA in this kit. DNase treatment of the wells of the filter plate directly is not recommended. To remove all DNA from the final RNA product, make the RNA solution 10 mM TrisHCl, pH 7.6, 2.5mM MgCl₂, 0.5mM CaCl₂ by using a 10X DNase I buffer concentrate (not supplied), add 1-2 U of RNase-free DNase I (not supplied), incubate for 15 min. at 37°C and inactivate the DNase I by heating the sample to 75°C for 10 min. If EDTA will not affect your downstream application it is recommended to first make the solution 5 mM with EDTA. This will help protect the RNA during the enzyme inactivation. For most applications, this provides a sufficiently clean RNA. However, the Xpress EZ-RNA 96 Kit RNA cleanup protocol can be followed when absolutely all nucleotides, EDTA and salts must be removed.

TROUBLESHOOTING

Problem	Comments and Suggestions
Little or no RNA eluted	All buffers must be at room temperature. Ensure that vacuum draws all liquid through filter at each step. Perform RNA extraction with no interruptions. Measure final elution volume- ensure adequate final elution from final centrifugation steps.
Filters clog	Too much RNA/cells used. Reduce sample size. Be careful when taking supernatant from spheroplast lysate.
Filters tear/plates break	Reduce centrifugation speed.
Degraded RNA/High Interwell variation	Consider finding sources of RNase contamination. Inadequate vacuum during washing.
RNA performs poorly	Ensure that that plate is completely dry and that all traces of ethanol have been removed before final elution step (increase drying time to 10 min).

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