

CytosALL™ RNA Extraction Kit

PRODUCT NUMBER:	AB-4410
STORE:	+4°C (Reagent) and ambient (Tips)
SHIP:	Ambient
KIT CONTENTS:	- 40 mL CytosALL Reagent - 96 x 1 mL Pipette Tips - 96 x 200 µl Filter Tips

INSTRUCTIONS FOR USE

The CytosALL™ RNA Extraction Kit is used to prepare RNA for direct use in downstream applications such as RT-PCR and QRT-PCR. The reagent lyses the cytoplasmic membrane without disrupting the nuclei. Filtration tips are used to remove nuclei (and genomic DNA) from the sample.

Cultured cells are lysed using a single-step procedure that releases RNA. The protocol is recommended for adherent cells (grown in a monolayer) or cells grown in suspension. Each filter tip can be used for up to 5×10^5 cells. Sample size can be scaled down as needed.

- 1) Prepare cell pellet:
 - a. Harvest and pellet 5×10^5 cultured cells. Place on ice.
 - b. Wash once with cold phosphate buffered saline (PBS).
 - c. Remove and discard supernatant.
- 2) Prepare lysis reagent on ice:
For each 5×10^5 cell sample, add 3 µl RNase inhibitor (20-40 units/µl) to 300 µl CytosALL Reagent.
NOTE: RNase Inhibitor is not included in the kit, but may be purchased separately (item # AB-1296/B).
- 3) Prepare the lysate(s) as follows. For optimal results complete processing each sample prior to beginning the next, as prolonged incubation may lead to nuclear lysis:
 - a. Add 300 µl cold lysis reagent (from step 2) to the pellet.
 - b. Resuspend the pellet by pipetting eight to ten times or until the pellet is completely disrupted.
 - c. Immediately filter the lysate.

For Research Purposes Only

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- 4) Filtration of the lysate may be performed using a manual or electronic pipettor. In either method air is aspirated into the tip prior to the lysate. This column of air aids in the filtration process.

A) Sample Filtration – Manual Pipette:

- a. Firmly attach the 1 mL tip provided with the kit to a 1 mL pipette.
- b. Depress the plunger down to expel all the air.
- c. Release the plunger ~3/4 of the full distance to aspirate ~ 700 µL air.
- d. While still holding the plunger down, place the tip in the sample tube and aspirate the 300 µL of lysate.
- e. Attach the 200 µL Filter tip (provided with the kit), to the end of the 1 mL tip. Attach the tip securely but do not use excessive force as this may impede filtration.
- f. To expel the sample, press the plunger all the way down and hold until the lysate is filtered (this will take several seconds). Collect the filtered lysate in a new pre-chilled nuclease free tube. Keep the filtered lysate on ice.

B) Sample Filtration – Electronic Pipettor (Thermo Scientific FinnpiPETTE® Novus 1 mL Pipettor)

- a. Program the pipettor as follows:
 - i. Select MENU from the lower left corner.
 - ii. Highlight PROGRAM and press OK.
 - iii. Scroll to the program number to be written (1 – 9) and press OK.
 - iv. Select EDIT from the lower right corner.
 - v. Scroll to SEQ ASPIRATE and press OK (the number of steps will flash in the display).
 - vi. Scroll to 2 and press OK.
 - vii. Scroll to set the first VOL to 700 µL and press OK.
 - viii. Set the second VOL to 300 µL and press OK.
 - ix. TOTAL 1000 µL will be displayed.
 - x. The ASPIRATE speed will flash in the upper left corner of the display.
 - xi. Scroll to set the ASPIRATE speed to 8 and press OK.
 - xii. The DISPENSE speed will flash in the upper left corner of the display.
 - xiii. Scroll to set the DISPENSE speed to 3 and press OK.
- b. Firmly attach the 1 mL tip provided with the kit to the pipettor.
- c. Press trigger to aspirate 700 µL of air.
- d. Place the tip in the lysate containing tube and press the trigger a second time to aspirate 300 µL of the lysate.
- e. Attach a 200 µL Filter tip to the end of the 1 mL tip. Attach the tip securely but do not use excessive force as this may impede filtration.
- f. To expel the sample, press the trigger a final time (filtration will take several seconds). Collect the filtered lysate in a new pre-chilled nuclease free tube. Keep the filtered lysate on ice.

- 5) Aliquot and store the undiluted lysate at -20°C or -80°C (for up to two weeks) or use directly in desired application.
Further RNA purification is not required for downstream applications, such as RT-PCR and QRT-PCR.
- 6) Some applications may require dilution of the lysate. Table 1 (overleaf) provides general recommendations for dilution; the optimal dilution will need to be determined experimentally.

	Suggested Dilution Range	Volume to add to 20 µl RT reaction	Volume to add to 25 µl PCR reaction	Cell equivalents
1-step RT-PCR	1:2 – 1:1,000	-	1 µl diluted sample	1 – 1,000
1-step QRT-PCR	1:75 – 1:15,000	-	5 µl diluted sample	<0.5 –100*
2-step RT-PCR	Neat – 1:20,000	1 µl diluted sample	1 – 2.5 µl RT reaction	1 –400
2-step QRT-PCR	1:5 – 1:50,000	1 µl diluted sample	1 –5 µl RT reaction	<0.5 – 50*

*For linear amplification (when absolute quantification is desired) use 0.5-10 cells for 1-step QRT-PCR or 1-10 cells for 2-step QRT-PCR.

Warranty

This product (“Product”) is warranted to operate or perform substantially in conformance with published Product specifications in effect at the time of sale, as set forth in the Product documentation, specifications and/or accompanying package inserts (“Documentation”) and to be free from defects in material and workmanship. Unless otherwise expressly authorized in writing, Products are supplied for research use only. No claim of suitability for use in applications regulated by FDA is made. The warranty provided herein is valid only when used by properly trained individuals. Unless otherwise stated in the Documentation, this warranty is limited to one year from date of shipment when the Product is subjected to normal, proper and intended usage. This warranty does not extend to anyone other than the original purchaser of the Product (“Buyer”).

No other warranties, express or implied, are granted, including without limitation, implied warranties of merchantability, fitness for any particular purpose, or non infringement. Buyer’s exclusive remedy for non-conforming Products during the warranty period is limited to replacement of or refund for the non-conforming Product(s).

There is no obligation to replace Products as the result of (i) accident, disaster or event of force majeure, (ii) misuse, fault or negligence of or by Buyer, (iii) use of the Products in a manner for which they were not designed, or (iv) improper storage and handling of the Products.

Ordering Information

AB-4410/A	CytosALL™ RNA Extraction Kit	40 ml (96 rxns)
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Related Products

RNase Inhibitor:	AB-1296 RNase Inhibitor (Porcine)
Plates:	ABgene® SuperPlate™ ABgene® PCR Plate
cDNA Synthesis Kit:	Verso™ cDNA Synthesis Kit
QPCR Kits:	ABsolute™ Blue QPCR Mix ABsolute™ Blue QPCR SYBR Green Mix
QRT-PCR Kits:	Verso™ 1-Step QRT-PCR Kit Verso™ SYBR® Green 1-Step QRT-PCR kit Verso™ 2-Step Kit Verso™ SYBR® Green 2-Step Kit

These products are available under multiple references and format to fit most of the instruments of the market. Please refer to our website www.abgene.com or contact us directly for more information (details below).

Troubleshooting

For troubleshooting, see www.abgene.com/troubleshoot.asp or contact Thermo Fisher Scientific (ABgene) TechSupport at abgene.techsupport@thermofisher.com

UK TechSupport, call +44 (0) 1372 840 410

For all other regions, please contact your local Thermo Fisher Scientific (ABgene) office / distributor.

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