Product Information

Extract-N-Amp™ Cellular RNA Lysis Buffer

Optimized for rapid cell lysis and RNA stabilization

XNACRL

Product Description

The Extract-N-Amp™ Cellular RNA Lysis buffer (XNACRL) is a single working solution that allows for rapid, room-temperature extraction and stabilization of RNA from a range of cells without heat, agitation or clean up. This is a simple and robust method for preparing cell lysate and stabilizing the extracted RNA for gene expression analysis directly from the cell lysate without the need for purification step prior to analysis. It reduces significant handling time and multiple handling steps, thereby reducing the risk for sample loss and contamination. It performs equally well on both adherent and non-adherent mammalian cells in the range of 5-100,000 cells. XNACRL produced RNA lysate extracts are stable for at least 6 months at −20 °C and can be stored in deep storage for at least a year (−80 °C).

Briefly, 5-100,000 cells are counted and pelleted (washing cells in a salt solution is optional). XNACRL (Extract-N-Amp™ Cellular RNA Lysis) buffer is added to lyse cells and inactivate RNase, resulting in stabilized, exposed RNA. It is a standalone reagent suitable for compatibility with RT-qPCR, single-cell analysis and NGS library prep for contamination-free RNA detection and analysis. It can be paired with DNase I to ensure removal of DNA for more sensitive processes.

Features

- Rapid room temperature process
- Effective with various mammalian cell types
- Extracts total RNA for downstream applications
- May be used to extract miRNA
- Single-solution process

Applications

XNACRL extracts can be used for:

- miRNA Analysis
- qRT-PCR
- Next Generation Sequencing
- Single-Cell Analysis

Unit Definition

One unit incorporates 10 nmol of total deoxyribonucleoside triphosphates into acid precipitable DNA in 30 minutes at 74 °C.

Materials and Reagents Required (Not included, see Product Ordering)

- Cultured mammalian cells
- Dulbecco's Phosphate Buffered Saline (PBS, D8662), or Hank's Balanced Salt Solution (HBSS, H9269), or equivalent balanced salt solution
- Real-time qPCR thermal cycler
- PCR tubes, 96-well plates, or glass capillary tubes recommended by the thermal cycler manufacturer.
- Magnesium chloride solution, 25 mM, (M8787).
- PCR primers and probe(s)
- RT-PCR (QR0200), and other downstream reagents
- Optional: AMPD1, DNase treatment

Precautions and Disclaimer

This product is for R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

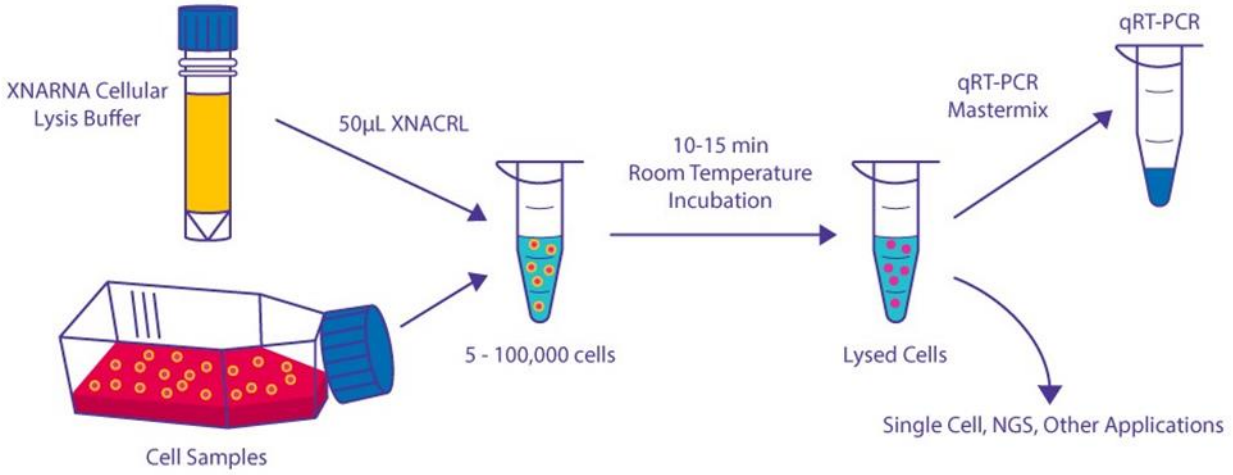
Upon receipt, store all reagents at <−20 °C. Recommended, as repeated freeze-thaw cycles of this product may adversely affect its function.

Directions for Use

This protocol is written for cell cultures grown in culture flasks, 96-well or 384-well tissue culture plates. While up to 100,000 cells may be used per extraction, results vary depending on cell type. A pilot experiment may be recommended to ensure that the number of cells to be used is in the linear range of detection.

For best results, lyse cells before they reach maximum density or confluency. If cells were recently seeded, make sure they are fully attached before removing medium and washing.

All steps are carried out at room temperature unless otherwise noted. Equilibrate XNACRL Extraction Solution to room temperature prior to use.



Cell Preparation-Adherent Cells in 96 or 384-well plates

1. Count (or estimate) the number of cells per well. Greater than 100,000 per well may inhibit RT-qPCR.

   Note: Use <10,000 input cells to obtain best results from optional DNase treatment.
2. Carefully remove the growth medium by aspiration. Be careful not to disturb the cell monolayer. Proceed to step 2.

   Note: (Optional) Wash the cells to remove trace amounts of growth media by gently adding 100 µL PBS, HBSS, or equivalent balanced salt solution without disturbing the cell monolayer.
3. If wash was performed, carefully remove as much wash solution as possible by aspiration before proceeding to step 2.

Cell Preparation-Suspension cells or cells released from adherent cultures

1. If using cells from an adherent culture of more than 100,000 cells, follow standard detachment procedures. If trypsin, Accutase® solution (A6964), or similar cell detaching solution is used, inactivate it with serum or serum-containing medium before proceeding.
2. Transfer up to 100,000 detached or suspension cells to a new vessel and pellet by centrifugation at 125 x *g* for 5 minutes.

   Note: Use <10,000 input cells to obtain best results from optional AMPD1 DNase treatment.
3. Carefully aspirate the media, avoiding the cell pellet.

   Optional: Wash the cells in PBS (D8662), HBSS (H9269), or an equivalent balanced salt solution by resuspending the cells and spinning at 125 x *g* for 5 minutes.
4. If wash was performed, aspirate the wash solution being careful to avoid the cell pellet. Proceed to step 2.

RNA Extraction

1. Thaw vial of XNACRL at room temperature. Once thawed, store on ice until ready for use.
2. Add 50 µL of XNACRL Extraction Solution to each well containing cells and incubate at room temperature (20-25 °C) for 10 minutes.
3. Lysates should be placed on ice immediately after incubation while preparing reverse transcription reactions or other downstream application. Stored at < −20 °C for up to 6 months and ≤ 80 °C for long term storage.

Reverse Transcription qPCR Setup using Extract-N-Amp™ Cellular RNA Lysis Buffer XNACRL and QR0200 Quantitative RT-PCR Ready Mix

If performing RT-qPCR, reagents should be completely thawed, kept on ice and gently mixed before use.

Primer Design & Optimization

Use primer design software to design primers that minimize complications from genomic DNA contamination, primer-dimers, non-specific hybridization, and secondary structures. The preferred amplicon for qPCR is 100-150 bp in length, and should not exceed 500 bp.

Intron-spanning primers are recommended to avoid potential false-positive results associated with amplification of contaminating genomic DNA.

Cycling parameters and primer and probe concentrations should be optimized for each experiment and will depend on the system being used. Typical final primer concentrations are ~0.5 µM each. Generally, dual-labeled fluorescent probes are used at half the final concentration of that used for the primers (For example: ~0.25 µM). Lower primer concentrations may decrease primer-dimer formation and nonspecific product formation.

Controls

We recommend using purified total RNA (2-50 ng) as a positive control, Extraction Solution as a negative control, and no-RT controls to measure false positive results from the amplification of genomic DNA.

Use the following table to prepare an appropriate volume of RT master mix.
Note: The RT master mix is formulated to accommodate 5-8 µL of cell extract per 20 µL reaction and 10-16 µL of cell extract per 50 µL reaction. Adjust water volume accordingly when altering extract volume. Master mix volume may also be scaled to accommodate different RT reaction volumes.

Assemble Reaction Mix

Reagent	Final Concentration	Amount per 20 µL reaction
2x qRT-PCR Ready Mix	0.05 U/µL Taq 10 mM Tris-HCl 50 mM KCl 3 mM $MgCl_2$ 0.2 mM each dNTP 0.5 U/µL RNase inhibitor	10 µL
M-MLV Reverse Transcriptase	1 U/µL (dilute in 10x PCR buffer if necessary)	0.1 µL
$MgCl_2$	Supplement to increase beyond 3.0 mM	Variable
Primers	0.1-0.5 µM (Optimizable)	Variable
Probe	0.25 µM	Variable
RNA Template	Variable	Variable
Nuclease-Free Water		Fill up to 20 µL

Add Template

1. For a 20 µL reaction, dispense 12-15 µL aliquots of RT Master mix into nuclease-free 0.2 mL PCR tubes or wells of a multi-well PCR plate.
- Add 5-8 µL of cell extract per reaction to bring final volume to 20 µL and mix until homogenous by pipetting up and down.
2. For a 50 µL reaction, dispense 34-40 µL aliquots of RT mastermix into nuclease-free 0.2 mL PCR tubes or wells of a multi-well PCR plate.
- Add 10-16 µL of cell extract per reaction to bring final volume to 50 µL and mix until homogenous by pipetting up and down.
3. Perform qRT-PCR with cycling parameters like above. Adjust for primer-probe, product, size, sample contents, etc.

Amplify

A suggested thermocycling protocol for 100-600 bp fragments:

Reverse Transcription		42-44 °C	30 min
Initial denaturation		94 °C	2 min
40 cycles	Denaturation	94 °C	15 seconds
	Annealing, extension, detection	60 °C or 5 °C below lowest primer T_m	1 min
Hold (Optional)		4 °C	∞

Amplification parameters will vary depending on primers, template, and instrument used.

Troubleshooting Guide

Problem	Suggestions	Solution
No RT-qPCR product (signal) is observed, or RT-qPCR product is detected late in qPCR	RNA was degraded before adding Extraction Solution	Minimize cell-handling time, and store cells on ice until just before adding the extraction solution.
	RNA was degraded during or after extraction.	The Extraction Solution contains components to inactivate RNases, but extremely high levels of native RNases may not be completely inactivated. Do not allow cell extracts to sit at room temperature for >15 minutes. If RNA degradation occurs, reduce the number of input cells per extraction and/or add RNase inhibitor to the Extraction Solution.
	Target message is not expressed at a high enough level.	Positive controls (For example: B-actin, GAPDH, etc.) will help diagnose the cause of failed amplification results. Conversely, negative controls will help identify false positives.
	Low cell numbers or low target copy numbers were used per extraction.	Rare messages and low cell numbers per reaction will result in linear qPCR detection as late as 40 cycles. If detection at such late cycles is of concern, perform a standard curve (For example: ten-fold dilutions of 10^6 to 10 input cells) to confirm detection linearity.
	RT-qPCR product is too long.	The best results are obtained when RT-qPCR products are between 100-150 bp and do not exceed 500 bp.
	Primer or probe design or concentration, or reaction conditions are suboptimal.	Refer to our qPCR Technical Guide(ref 4) or "A-Z of Quantitative PCR"(ref 3) for information on primer, probe, and reaction condition optimization.

Multiple RT-PCR products	Nonspecific priming due to primer or probe design, concentration, or suboptimal reaction conditions.	Refer to our qPCR Technical Guide[ref 4] or "A-Z of Quantitative PCR"[ref 3] for recommendations on primer, probe, and reaction condition optimization.
	Genomic DNA contamination in the RT reaction.	Design primers such that the 3'-end of one span an exon-exon junction or the pair flanks a large intron to eliminate amplification from genomic DNA.
	JumpStart™ *Taq* antibody is not working correctly.	Do not use DMSO or formamide with Jumpstart™ *Taq* Ready Mix. It can interfere with the enzyme-antibody complex. Other co-solvents, solutes (For example, salts) and extremes in pH or other reaction conditions may reduce the affinity of the JumpStart Taq antibody for Taq polymerase and thereby compromise its effectiveness.
Negative control (water + RT mix) shows RT-qPCR product (signal)	Reagents or reactions have been contaminated with PCR product from previous reactions.	Clean the area in which RT-qPCR is set up with 10% bleach, then repeat experiment being careful not to contaminate reactions. Replace reagents if contamination is still a problem.
No RT control shows RT-qPCR product (signal)	Residual genomic DNA is amplified during qPCR	Redesign primers such that the 3'-end of one span an exon-exon junction or the pair flanks a large intron. The DNase treatment protocol is optimized to degrade genomic DNA from < 10,000 cells. If higher cell numbers are being used, consider reducing input cell number, doubling the amount of DNase added per extraction, increasing the extraction incubation step to 20 minutes, and/or increasing the incubation temperature to 37 °C.

References

1. Lovatt, A., et al. Validation of Quantitative PCR Assays, BioPharm, March 2002, p. 22-32.
2. Bustin, S.A., Quantification of mRNA using realtime reverse transcription PCR (RT-QPCR): trends and problems, J. Molecular Endocrinology, 29, 23-29 (2002).
3. Bustin, S.A., A-Z of Quantitative PCR. International University Line (IUL), La Jolla, CA, USA, 2004.
4. Sigma-Aldrich qPCR Technical Guide (http://www.sigmaaldrich.com/life-science/molecular-biology/pcr/quantitative-pcr/qpcr-technical-guide.html).

Product Ordering

Description	Catalogue Number
Quantitative RT-PCR Ready Mix	QR0200
Custom ordered primers specific to gene target	OLIGO
Magnesium chloride solution	M8787
Accutase® solution	A6964
Hank's Balanced Salt Solution (HBSS)	H9269. H9394
M-MLV Reverse Transcriptase	M1302
Trypsin solution from porcine pancreas	T4674, T4549,T4424
Trypsin-EDTA solution	T4049, T3924, T4174,T4299
Dulbecco's Phosphate Buffered Saline (PBS)	D8662
Water, Microbial DNA-free	MBD0025
Nuclease-Free Water, for Molecular Biology	W4502
Nuclease-free water	W1754
DNaseI	AMPD1
Bst Max DNA Polymerase	SRE0113

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