SOP TITLE:	VERSION:	
PM-PD-SOP3.5 RNA extraction from cells in PeptiMatrix hydrogels	1	
AUTHOR & RESEARCH TEAM: Mikayla Shelton (MES) - Product Development	STATUS: Approved	

SOP REVIEWED AND APPROVED BY JWC (MAY 27, 2025, 1:46 PM +0100)

SECTION ADDED: Mar 5, 2025, 3:52 PM +0000
LAST EDIT: Mar 5, 2025, 4:00 PM +0000

1. BACKGROUND

This SOP covers 2 separate protocols for extracting RNA from cells encapsulated in hydrogels, Method A using a commercial kit from Qiagen (RNeasy® Mini Kit) and Method B that does not require a kit. Depending on the hydrogel functionalisation and kit/reagent availability, you must consider which method is best (see Table 1). The general steps of both protocols include homogenization/lysis of cells or tissues, extraction of RNA, precipitation, and resuspension. The purified RNA is ready for use in downstream applications such as RT-PCR and real-time RT-PCR, cDNA synthesis and microarrays.

2. RISK ASSESSMENT

Please refer to PM-RA6.1 for a full risk assessment of the following protocol. Ensure that you fully understand the potential risks and have received the appropriate training before starting any work.

SECTION ADDED: Mar 5, 2025, 3:52 PM +0000
LAST EDIT: May 13, 2025, 1:48 PM +0100

Product name	Product code	Method A compatibility	Method B compatibility
PeptiMatrix Core	PMCORE	Y	Y
PeptiMatrix RGD	PMRGD	Y	Y
PeptiMatrix IKVAV	PMIKVAV	N	Y
PeptiMatrix Plus	PMPLUS	Y	Y

Table 1: Compatibility table of each type of gel functionalisation with both methods of RNA extraction

SECTION ADDED: Feb 17, 2025, 11:28 AM +0000
LAST EDIT: Mar 5, 2025, 4:00 PM +0000

Method A - RNeasy® Mini Kit

Note: RNeasy Mini Spin Columns have a maximum binding capacity of 100µg RNA and recommend a maximum amount of starting material of 1 x 10^7 cells for efficient lysis to prevent cell debris interfering with the column. If you have more starting material than this, consider splitting sample into 2x spin columns to obtain an optimal RNA yield and purity, then after the final stage pool RNA together or use a further concentration step.

3. MATERIALS

- Certified chemical fume hood
- Cells in gels for RNA extraction (typically combine 4-20x wells for a good yield)
- Centrifuge that reaches 8,000x g
- Vortex
- RNeasy® Mini Kit (Qiagen #74104)
- Molecular biology grade Ethanol 100%
- RNase free water
- RNAse free 1.5mL microcentrifuge tubes
- RNAse free pipette tips (p10, p200, p1000)
- Pipettes (p10, p200, p1000)
- -80°C freezer
- Ice
- RNase Decontamination Solution e.g. RNaseZap™ or RNase AWAY™
- Optional: 14.3 M β-mercaptoethanol (β-ME)*

4. METHODS

Carry out all steps in chemical fume hood

Preparation before extraction

1. Clean chemical fume hood to remove potential ribonuclease enzymes which can rapidly degrade RNA using decontamination solution.
2. Prepare 70% ethanol with 100% ethanol + RNase free water in RNAse free centrifuge tube
3. Prepare a working solution of Buffer RPE concentrate by adding 4 volumes of 100% ethanol as indicated on the bottle.

*Optional step: If purifying RNA from cell lines rich in RNAses, it is recommended to add 10 µl β-mercaptoethanol (β-ME) per 1mL Buffer RLT before use. Buffer RLT containing β-ME can be stored at room temperature for up to 1 month.

RNA extraction (See Figure 1)

Complete all steps in chemical fume hood:

1. Remove plate from incubator and remove all media
2. Add 100µL RLT Buffer to each well of gel and pipette up and down vigorously to lyse cells.
3. Combine wells into an RNAse free tube and vortex for 1 min.
4. Add equal volume of 70% ethanol and mixed well by pipetting.
5. Add total volume to RNeasy mini spin column in a collection tube and spin 8000x g 15 sec. Spin column can hold up to 700 µl volume, so can do multiple spins if total volume is over 700 µl. Discard flow through each time to phenol waste.
6. Once all sample volume has moved through spin column, move spin column to a new RNase free collection tube.
7. Add 700 µL RW1 buffer then centrifuge at 8000x g 15 sec. Discard flow through.
8. Add 500 µL RPE buffer and leave to stand for 1 min, then spin 8000x g 15 sec. Discard flow through. Repeat this step at least 3x times to remove guanidine salts.
9. Add 500 µL RPE buffer then centrifuge at 8000x g for 2 min. Discard flow through.
10. Centrifuge at 8000x g for 1 min to dry membrane.
11. Place spin column into an RNAse free tube. Add 30-50 µL RNAse free water and leave for 5 mins.
12. Centrifuge at 8000x g for 1 min to elute RNA.

SECTION ADDED: Mar 5, 2025, 3:33 PM +0000
LAST EDIT: Mar 5, 2025, 3:51 PM +0000

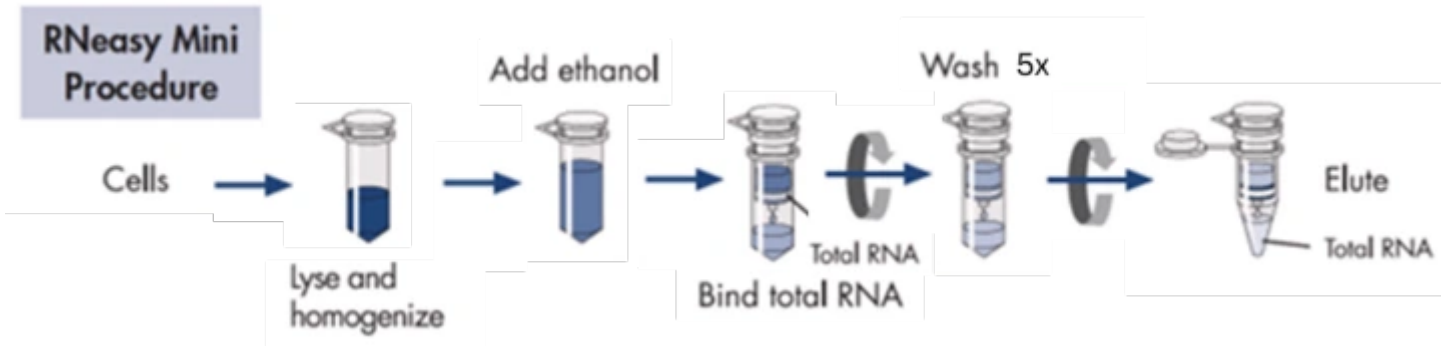

Figure 1: Schematic of the different steps in RNA extraction Method A (adapted from the RNeasy Mini Handbook)

SECTION ADDED: Mar 5, 2025, 3:46 PM +0000
LAST EDIT: Mar 5, 2025, 4:02 PM +0000

Method B - Trizol® + chloroform method

This protocol uses an all- in- one acid guanidinium thiocyanate- phenol solution TRIzol ® and isopropanol for the precipitation step.

3. MATERIALS

- Certified chemical fume hood
- Cells in gels for RNA extraction (typically combine 4-20x wells for a good yield)
- Temperature controlled centrifuge that reaches 4°C and 12,000x g

- TRIzol® reagent
- Chloroform/isoamyl alcohol (49:1)
- Molecular biology grade 2-Propanol >=99.5%
- Molecular biology grade Ethanol 100%
- RNase free water
- RNAse free 1.5mL microcentrifuge tubes
- RNase free 15mL centrifuge tubes
- RNAse free pipette tips (p10, p200, p1000)
- Pipettes (p10, p200, p1000)
- -80°C freezer
- Ice
- RNase Decontamination Solution e.g. RNaseZap™ or RNase AWAY™

4. METHODS

Preparation before extraction

1. Turn centrifuge to 4°C
2. Clean chemical fume hood to remove potential ribonuclease enzymes which can rapidly degrade RNA using decontamination solution.
3. In chemical fume hood, prepare 75% ethanol with 100% ethanol + RNase free water in RNAse free centrifuge tube

RNA extraction (see Figure 2)

Complete all steps in chemical fume hood:

1. Remove plate from incubator and remove all media
2. Add 1mL TRIzol® reagent per 1 x 10^7 cells (~100 µl per well of a 96 well plate)
3. Leave sample for 5 minutes at room temperature to allow dissociation of nucleoprotein complexes
4. Combine sample into RNAse free microcentrifuge/centrifuge tube
5. Add 0.2mL chloroform/isoamyl alcohol per 1mL TRIzol®
6. Shake vigorously by hand for 10 seconds
7. Incubate samples on ice for 3 minutes
8. Centrifuge samples for 15 minutes 12,000x g at 4°C to separate RNA from rest of lysate. The sample will have 3 layers (see Figure 1): clear aqueous layer = contains RNA, fluffy white interphase layer = cell debris, red organic layer = contains phenol and chloroform
9. Transfer top aqueous layer to new RNAse free tube (be careful to not collect any of the interphase layer)
10. Add an equal volume of 2-propanol
11. Incubate for 1 hour at -20°C or overnight at -80°C for an increased RNA yield
12. Centrifuge for 20 minutes 10,000x g at 4°C
13. Discard supernatant, leaving a gel-like white pellet of total RNA at the bottom of the tube
14. Wash the RNA by resuspending the pellet in 0.5-1mL 75% ethanol and vortexing for a few seconds
15. Centrifuge for 5 minutes 10,000x g at 4°C
16. Discard supernatant (repeat wash step if desired)
17. Resuspend RNA pellet in RNAse free water

SECTION ADDED: Feb 17, 2025, 11:28 AM +0000
LAST EDIT: Mar 5, 2025, 3:48 PM +0000

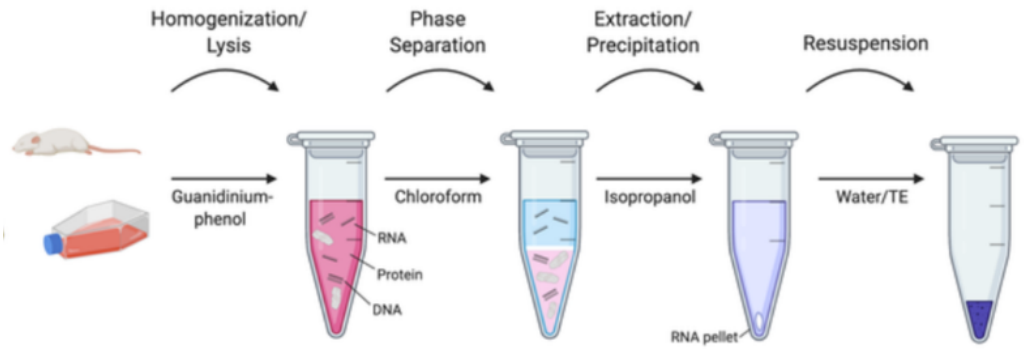

Figure 2: Schematic of the different steps in RNA extraction (taken from Addgene: https:// www.addgene.org/ protocols/ kit- free- rna- extraction/)

SECTION ADDED: Feb 17, 2025, 11:28 AM +0000
LAST EDIT: Mar 5, 2025, 3:48 PM +0000

5. DISPOSAL

1. Dispose of cell culture medium by pouring into waste pot/aspirating into vacuum trap containing Chemgene (or appropriate laboratory disinfectant). Leave in Chemgene for at least one hour.
2. **DO NOT** add bleach or acidic solutions to the sample waste. RLT buffer (Method A) and Trizol® (Method B) contains guanidine thiocyanate. Guanidine salts can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/ v) sodium hypochlorite.
3. Dispose of waste containing phenol + chloroform according to the establishment's Biological and Chemical Waste Handling procedure.

User Initials Legend

JWC Johnathan Curd
MES Mikayla Shelton