



ZYMO RESEARCH

RNA
Purification
Made Simple

Quick-RNA™ Miniprep Plus Kit

RNA from any sample

Highlights

- Spin-column purification of total RNA (including small/microRNAs) from any sample including cells, solid tissue, biological liquids, environmental samples, swabs and any sample in DNA/RNA Shield™
- DNA/RNA Shield™ and Proteinase K is included for unique preservation and lysis technology.
- RNA is eluted in two separate fractions and is ready for Next-Gen Sequencing, RT/qPCR, etc. *DNase I is included.*

Catalog Numbers:

R1057T, R1057, R1058



Scan with your smart-phone camera to view the online protocol/video.



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Product Contents

Quick-RNA™ Miniprep Plus Kit	R1057T (10 prep)	R1057 (50 prep)	R1058 (200 prep)
RNA Lysis Buffer	10 ml	50 ml	100 ml (x2)
RNA Prep Buffer	5 ml	25 ml	100 ml
RNA Wash Buffer ¹	16 ml (ready-to-use)	24 ml (concentrate)	48 ml (x2)
DNase/RNase-Free Water	1 ml	6 ml	30 ml
DNase I ² (lyophilized)	50 U	250 U	250 U (x4)
DNA Digestion Buffer	0.8 ml	4 ml	16 ml
DNA/RNA Shield™ (2X concentrate)	5 ml	25 ml	125 ml
PK Digestion Buffer	1 ml	5 ml	20 ml
Proteinase K ³ (lyophilized) & Storage Buffer	5 mg	20 mg	20 mg (x4)
Spin-Away™ Filters	10	50	200
Zymo-Spin™ IIICG Columns	10	50	200
Collection Tubes	20	100	400
Instruction Manual	1 pc	1 pc	1

Storage Temperature - Store all kit components (i.e., buffers, columns) at room temperature.

Before use:

1 Add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **RNA Wash Buffer** concentrate (R1057) or 192 ml 100% ethanol (208 ml 95% ethanol) to the 48 ml **RNA Wash Buffer** concentrate (R1058). **RNA Wash Buffer** (R1057T) is supplied ready-to-use and does not require the addition of ethanol.

2 Reconstitute lyophilized **DNase I** with **DNase/RNase-Free Water**, mix by gentle inversion and store frozen aliquots:

#E1009-A (250 U), add 275 µl water

#E1009-A-S (50 U), add 55 µl water

3 Add **Proteinase K Storage Buffer** to the lyophilized **Proteinase K**, 20 mg, see Buffer Preparation, page 4. Store frozen aliquots.

Specifications

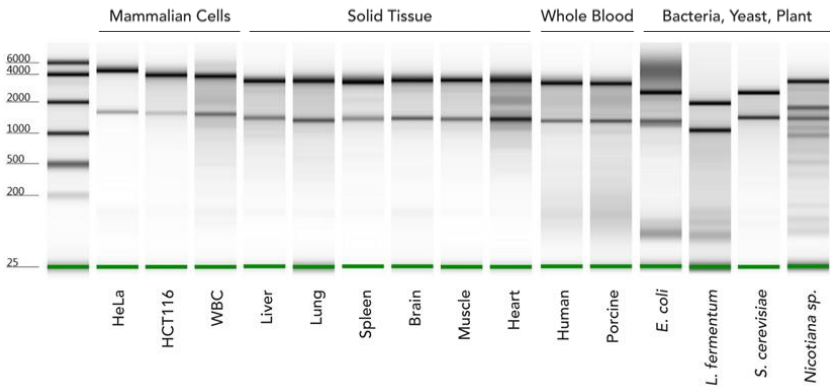
- **Sample Sources** – Any cells (animal, bacterial, blood cells, etc.), all tissues (tough-to-lyse, FFPE, etc.), blood, biological fluids, enzymatic reactions (e.g., DNase I treated) and samples in **DNA/RNA Shield™**.
- **Sample Preservation and Inactivation – DNA/RNA Shield™** lyses cells, inactivates nucleases and infectious agents (e.g., virus, pathogens) and is ideal for safe sample storage and transport at ambient temperatures (page 9).
- **Size** – Total RNA including small/microRNAs (≥ 17 nt).
- **Purity** – A_{260}/A_{280} & $A_{260}/A_{230} > 1.8$. RNA is ready for Next-Gen Sequencing, RT/qPCR, etc.
- **Binding Capacity** – **Zymo-Spin™ IIICG Column (green)** yield up to 100 μg RNA.
- **Compatibility** – For samples stored in preservation reagents: **DNA/RNA Shield™**, RNAprotect®, Allprotect®, Universal transport medium/viral transport medium (UTM®/VTM®), PAXgene® and RNAlater™.
- **Elution Volume** – ≥ 50 μl **DNase/RNase-Free Water**.
- **Equipment Needed** (user provided) – Microcentrifuge, vortex, heat block, water bath or incubator.

Product Description

The **Quick-RNA™ Miniprep Plus Kit** combines **Quick-RNA™** technology with the addition of **DNA/RNA Shield™**, a unique preservation and lysis technology, and **Proteinase K** to enable easy, reliable, and rapid isolation of RNA from any biological sample including any cells, all tissues, blood, and other biological fluids.

The procedure uses unique spin-column technology that results in high-quality total RNA (including small RNAs 17-200 nt) and is ready for Next-Gen Sequencing, RT/qPCR, hybridization, etc.

High-Quality RNA From Any Sample Type



High quality total RNA is isolated from various sample types including mammalian cells, solid tissue, whole blood, bacteria, yeast, and plant using the **Quick-RNA™ Plus** kits (Agilent 2200 TapeStation™).

Protocol

The protocol consists of: (I) Buffer Preparation, (II) Sample Preparation and (III) RNA Purification.

(I) Buffer Preparation

- ✓ Add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **DNA/RNA Wash Buffer** concentrate (R1057) or 192 ml 100% ethanol (208 ml 95% ethanol) to the 48 ml **RNA Wash Buffer** concentrate (R1058). **DNA/RNA Wash Buffer** (R1057T) is supplied ready-to-use and does not require the addition of ethanol.
- ✓ Reconstitute lyophilized **DNase I** with **DNase/RNase-Free Water**, mix by gentle inversion and store frozen aliquots:
#E1009-A (250 U), add 275 μ l **water**
#E1009-A-S (50 U), add 55 μ l **water**
- ✓ Reconstitute lyophilized **Proteinase K** at 20 mg/ml with **Proteinase K Storage Buffer** and mix by vortexing. Use immediately or store frozen aliquots:
#D3001-2-20 (20 mg), add 1.04 ml **buffer**
#D3001-2-5 (5 mg), add 0.26 ml **buffer**
- ✓ To prepare a 1X solution, add an equal volume of nuclease-free water (not provided) to the **DNA/RNA Shield™** (2X concentrate) (1:1) and mix well.

(II) Sample Preparation

- ✓ Perform all steps at room temperature and centrifugation at 10,000-16,000 x g for 30 seconds, unless specified.

Samples stabilized and stored in DNA/RNA Shield™ (cells, tissue, swab, etc.)

If frozen, thaw homogenized sample in **DNA/RNA Shield™** to room temperature (20-30°C). Mix well by vortex. Proceed to the appropriate procedure below based on sample type (omit the step involving the addition of DNA/RNA Shield™).

Cells¹

- For samples in **DNA/RNA Shield™**, add an equal volume of **RNA Lysis Buffer (1:1)**, mix well and proceed to purification, page 8.
- For cells, pellet by centrifugation (≤ 500 x g for 1 minute), remove the supernatant and resuspend the cell pellet in **RNA Lysis Buffer** (see table below). Proceed to purification, page 8.

Mammalian	Add RNA Lysis Buffer
$\leq 5 \times 10^6$	$\geq 300 \mu\text{l}$
$5 \times 10^6 - 10^7$	$\geq 600 \mu\text{l}$

Tough-to-Lyse Samples (bacteria, yeast, swab, soil², stool², solid tissue (animal, insect, plant², seed²))

- Tough-to-lyse samples (e.g., gram(+) bacteria, tissue, etc.), can be homogenized directly in $\geq 800 \mu\text{l}$ **DNA/RNA Shield (1X)**^{3,4} with a mortar/pestle, dounce, syringe, tissue grinder, or bead beating (recommended) with a homogenizer: high speed (e.g., MP Bio FastPrep-24, Bertin Precellys) or low-speed (e.g., Disruptor Genie).

Input	Gram(-) bacteria (optional; easy-to-lyse)	Gram(+) bacteria	Tissue
per prep	bacteria ($\leq 10^9$)	bacteria ($\leq 10^9$) yeast ($\leq 10^8$) swab, stool/soil (≤ 50 mg)	animal (high yield) (≤ 25 mg) animal (low yield) (≤ 50 mg) plant/seed, insect (≤ 200 mg)
lysis beads catalog #	0.5 mm and 0.1 mm; S6012	0.5 mm and 0.1 mm; S6012	2.0 mm; S6003
high-speed	30 sec	5-10 min	30-60 sec
low-speed	5-10 min	20-40 min	3-5 min

Continue to page 6 for tough-to-lyse samples.

1 For cells in suspension or in other liquids/media, add ≥ 3 volumes **RNA Lysis Buffer** to 1 volume liquid sample (3:1) and mix well. Proceed to purification, page 7.

2 For PCR inhibitor removal, use OneStep PCR Inhibitor Removal Kit (D6030).

3 For a 1X solution of **DNA/RNA Shield™**, see Buffer Preparation, page 4.

4 Solid tissues should be completely submerged in **DNA/RNA Shield™**, add as needed.

- For every 300 μ l sample, add 15 μ l **Proteinase K** and 30 μ l **PK Digestion Buffer**. Mix and incubate at room temperature (20-30°C). Recommended incubation time: \geq 30 minutes (homogenized) or 2-5 hours (non-homogenized). Optimization may be required.
- To remove particulate debris from homogenized tissue, centrifuge and transfer the cleared supernatant into a nuclease-free tube (not provided).
- Add an equal volume of **RNA Lysis Buffer** to the supernatant (1:1) and mix well. Proceed to purification, page 8.

FFPE Tissue

- Remove (trim) excess paraffin wax from \leq 25 mg FFPE tissue and transfer into a nuclease-free tube (not provided).
- Add 400 μ l **Deparaffinization Solution**¹ to the sample. Incubate at 55°C for 1 minute. Vortex briefly. Remove the **Deparaffinization Solution**.
- Add 95 μ l **DNase/RNase-Free Water**, 95 μ l **2X Digestion Buffer**¹, and 10 μ l **Proteinase K**. Mix well.
- Incubate at 55°C for 1 hour. Then incubate at 94°C for 20 minutes to de-crosslink the sample.
- Centrifuge to remove insoluble debris and transfer 200 μ l supernatant to a nuclease-free tube (not provided).
- Proceed to purification, page 8.

Blood Cells (mammalian, PBMCs, WBCs, etc.)

- For blood cells, buffy coat and pelleted PAXgene® or RNAlater™ samples, resuspend in **DNA/RNA Shield™** (1X)².

Blood Cells	Add DNA/RNA Shield™ (1X)
\leq 5 ml blood	\geq 300 μ l

- For every 300 μ l of sample, add 15 μ l **Proteinase K**.
- Mix and incubate at room temperature (20-30°C) for: \geq 30 minutes. Optimization may be required.
- After incubation, vortex sample and centrifuge at max speed for 2 minutes to pellet debris. Transfer 300 μ l of the cleared supernatant to a nuclease-free tube (not provided).
- Proceed to purification, page 8.

1 Deparaffinization Solution (D3067-1-20) and 2X Digestion Buffer (D3050-1-20) are sold separately.

2 For a 1X solution of **DNA/RNA Shield™**, see Buffer Preparation, page 4.

Whole Blood¹ (mammalian)

1. Add 200 μ l **DNA/RNA Shield**[™] (2X concentrate) directly to each 200 μ l of fresh/frozen blood sample and mix thoroughly².
2. For every 400 μ l of reagent/blood mixture, add 8 μ l **Proteinase K** and mix thoroughly. Incubate at room temperature (20-30°C) for 30 minutes.
3. Add an equal volume of isopropanol (1:1) and mix well. Proceed to purification, page 8.

¹ Up to 3 ml blood per prep can be processed (with reloading the column).

² To retain protein in the whole blood sample, omit step 2 and continue to step 3.

(III) Total RNA Purification

- ✓ Perform all steps at room temperature and centrifugation at 10,000-16,000 x g for 30 seconds, unless specified.
- 1. Transfer the sample lysed in **RNA Lysis Buffer** into a **Spin-Away™ Filter**¹ (**yellow**) in a **Collection Tube** and centrifuge to remove the majority of genomic DNA.

Save the flow-through!

- 2. Add 1 volume² ethanol (95-100%) to the flow-through (1:1) and mix well. Then transfer the sample into a **Zymo-Spin™ IICG Column**¹ (**green**) in a **Collection Tube** and centrifuge³. Discard the flow-through.
- 3. **DNase I**⁴ treatment (recommended)
 - (D1) Wash the column with 400 µl **RNA Wash Buffer** and centrifuge. Discard the flow-through.
 - (D2) In an nuclease-free tube, add 5 µl **DNase I** (1 U/µl)*, 75 µl **DNA Digestion Buffer** and mix. Add mixture directly into the column matrix.
 - (D3) Incubate the column at room temperature (20-30°C) for 15 minutes.
- 4. Add 400 µl **RNA Prep Buffer** to the column and centrifuge. Discard the flow-through.
- 5. Add 700 µl **RNA Wash Buffer** to the column and centrifuge. Discard the flow-through.
- 6. Add 400 µl **RNA Wash Buffer** and centrifuge the column for 1 minute to ensure complete removal of the wash buffer. Then carefully, transfer the column into a nuclease-free tube (not provided).
- 7. Add 100 µl **DNase/RNase-Free Water** directly to the column matrix and centrifuge.

Alternatively, for highly concentrated RNA use ≥ 50 µl elution.

The eluted RNA⁵ can be used immediately or stored frozen.

1 To process samples > 700 µl, columns may be reloaded.

2 To isolate large RNA species ≥ 200 nt, add 0.5 volume ethanol (95-100%) to the flow-through and mix well.

3 Optional: At this point, proteins can be purified from the flow-through (page 10).

4 Prior to use, reconstitute the lyophilized **DNase I** (Buffer Preparation, page 4). * Unit definition – one unit increases the absorbance of a high molecular weight DNA solution at a rate of 0.001 A₂₆₀ units/ml of reaction mixture at 25°C.

5 For complete removal of PCR (RT) inhibitors from plant, soil and fecal samples, use the OneStep™ PCR Inhibitor Removal Kit (D6030).

Appendices

Samples stabilized and stored in DNA/RNA Shield™

Recommended: **DNA/RNA Shield™** effectively lyses cells, inactivates nucleases and infectious agents and is ideal for sample storage/transport at ambient temperatures prior to nucleic acid purification.

Liquid samples: Mix an equal volume **DNA/RNA Shield™** (2X concentrate) and sample (1:1).

Solid samples: Submerge sample (not to exceed 10% (v/v or w/v) in **DNA/RNA Shield™** (1X).

Mix well/homogenize sample prior to storage. Samples in **DNA/RNA Shield™** can be stored at ambient temperature \geq month or long term at frozen temperature.

Samples in RNAProtect, All Protect, RNAlater, PAXgene, UTM/VTM, saline or PBS

✓ RNAProtect®, All Protect®: Add 3 volumes of **RNA Lysis Buffer** to 1 volume of liquid sample (3:1) and mix well and/or homogenize (e.g., see Tough-to-Lyse samples, page 5). Proceed to purification, page 8, step 2.

✓ RNAlater™: Add 1 volume of RNase-free water (or PBS) to 1 volume liquid sample (1:1) and mix. Then add 4 volumes **RNA Lysis Buffer** to 1 volume sample/water (or PBS) mixture (4:1). Mix again and proceed to purification, page 8, step 2.

Alternatively, remove the RNAlater™, then proceed with Sample Preparation according to the sample type.

✓ PAXgene®: Refer to manufacturer's instructions to remove the reagent and then proceed to Sample Preparation, Blood Cells, page 6.

✓ Swab samples in UTM®/VTM®, saline or PBS: Remove swab and add 3 volumes of **RNA Lysis Buffer** to 1 volume sample (3:1). Mix well and proceed to purification, page 8, step 2.

Optional: To inactivate, store and preserve at room temperature prior to purification, add 1 volume of DNA/RNA Shield™ (2X concentrate) to 1 volume of liquid sample (1:1) and mix well. Then proceed to Sample Preparation, Samples in DNA/RNA Shield™, page 5.

Liquids/Reaction Clean-up (DNase I treated RNA, in vitro transcriptions, etc.)

Add 150 μ l **RNA Lysis Buffer** to a \geq 50 μ l liquid sample (3:1) and mix well. Proceed to purification, page 8, step 2.

(Appendices continued)

Protein Purification: Acetone Precipitation of Proteins

- ✓ After the RNA binding to the column (page 8, step 2), the protein content in the flow-through can be purified:
- 1. Add 4 volumes of cold acetone (-20°C) to the flow-through (4:1) and mix.
- 2. Incubate the samples for 30 minutes on ice.
- 3. Centrifuge at max speed for 10 minutes. Discard the supernatant. Keep the pellet.
- 4. Add 400 µl ethanol (95-100%) to the protein pellet. Centrifuge at max speed for 1 minute. Discard the supernatant.
- 5. Air-dry the protein pellet for 10 minutes at room temperature.
- 6. Resuspend and vortex the pellet in a buffer appropriate for downstream application (e.g., SDS-PAGE sample loading buffer).

Purification of Small and Large RNAs into Separate Fractions

- ✓ This procedure is compatible with animal cell inputs ($\leq 10^6$) or purified RNA only.
- ✓ Perform all steps at room temperature and centrifugation steps at 10,000-16,000 x g for 30 seconds, unless specified.

1. Prepare adjusted **RNA Lysis Buffer** (as needed). Mix an equal volume of buffer and ethanol (95-100%).

Example: Mix 50 µl buffer and 50 µl ethanol.

2. Add 2 volumes of the adjusted buffer to the sample¹ and mix.

Example: Mix 100 µl adjusted buffer and 50 µl sample.

3. Transfer the mixture to the **Zymo-Spin™ Column**² and centrifuge. **Save the flow-through!**

4. **Small RNAs (17-200 nt)** are in the flow-through

- a. Add 1 volume ethanol and mix.

Example: Add 150 µl ethanol to 150 µl sample.

- b. Transfer the mixture to a **new column** and centrifuge. Discard the flow-through.

- c. Proceed with purification, page 8, step 4.

4. **Large RNAs (> 200 nt)** are retained in the column

- a. Proceed with purification, page 8, step 4.

¹ To minimize pipetting error, adjust the sample volume to 50 µl (minimum).

² To process samples > 700 µl, columns may be reloaded.

(Appendices continued)

Input Capacity and Average Total RNA Yield

Input	Average RNA Yield	Kit Capacity
Cells	10 µg (per 10 ⁶ cells)	Up to 10 ⁷
HeLa	15 µg	
High Yield Tissue ^{1 (mouse)}	≥ 30 µg (per 10 mg)	Up to 20 mg
Spleen	30-50 µg	
Liver	40-60 µg	
Low Yield Tissue ^{1 (mouse)}	≤ 30 µg (per 10 mg)	Up to 50 mg
Brain, Heart	5-15 µg	
Muscle	5-20 µg	
Lung	10-20 µg	
Intestine	10-30 µg	
Kidney	20-30 µg	
Whole Blood ²	(per 1 ml)	Up to 3 ml
Porcine	10-20 µg	
Human	2-10 µg	

1 Yield from tissue can vary due to other factors (i.e., organism type, physiological state and growth conditions).

2 Yield from blood can vary based upon collection, sample preparation, donor, age, and/or health conditions.

Ordering Information

Product Description	Catalog No.	Size
Quick-RNA™ Miniprep Plus Kit	R1057T	10 preps.
	R1057	50 preps.
	R1058	200 preps.
Individual Kit Components	Catalog No.	Amount
RNA Lysis Buffer	R1060-1-50	50 ml
	R1060-1-100	100 ml
RNA Prep Buffer	R1060-2-25	25 ml
	R1060-2-100	100 ml
RNA Wash Buffer (concentrate)	R1003-3-24	24 ml
	R1003-3-48	48 ml
DNase/RNase-Free Water	W1001-10	10 ml
	W1001-30	30 ml
DNase I Set (lyophilized) (250 U supplied with DNA Digestion Buffer, 4 ml)	E1010	1 set
DNA/RNA Shield™ (2X concentrate)	R1200-25	25 ml
	R1200-125	125 ml
PK Digestion Buffer	R1200-1-5	5 ml
	R1200-1-20	20 ml
Proteinase K (lyophilized) & Storage Buffer	D3001-2-5	5 mg
	D3001-2-20	20 mg
Spin-Away™ Filters	C1006-50-F	50
Zymo-Spin™ IIICG Columns	C1006-50-G	50
Collection Tubes	C1001-50	50

Complete Your Workflow

- ✓ For tough-to-lyse samples, use ZR BashingBead Lysis Tubes:

ZR BashingBead Lysis Tubes	
2.0 mm beads #S6003	Plant/animal tissue
0.1 + 0.5 mm beads #S6012	Microbes
0.1 + 2.0 mm beads #S6014	Microbes in tissue/insects

- ✓ For isolation of RNA from any sample:

Quick-RNA kits	
Microprep #R1050	From 1 cell and up
MagBeads #R2132/R2133	Automatable (Tecan, Hamilton, Kingfisher, etc.)

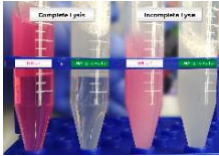
- ✓ For clean-up (purification) and concentration of any RNA sample. (e.g., from the aqueous phase of TRIzol[®] extractions) or from any enzymatic reaction (e.g., DNase I treated RNA):

RNA Clean & Concentrator kits	
Microprep #R1013-R1014	DNase I Set included
MagBeads #R1082	Automatable (Tecan, Hamilton, Kingfisher, etc.)

- ✓ For NGS:

Zymo-Seq RiboFree Total RNA Library Prep kit	
#R3000	12 preps
#R3003	96 preps

Troubleshooting Guide

Problem	Possible Causes and Suggested Solutions
<p>Precipitation, viscous lysate</p>	<p>Incomplete lysis and/or high-mass input:</p> <ul style="list-style-type: none"> - If precipitation occurs (upon adding ethanol to the lysate) or if the lysate is extremely viscous, increase the volume of DNA/RNA Shield™ and/or RNA Lysis Buffer to ensure complete lysis and homogenization until lysate is transparent (see image). 
<p>Low purity (A_{260}/A_{230} nm, A_{260}/A_{280} nm)</p>	<p>Sample handling:</p> <ul style="list-style-type: none"> - Ethanol and/or salt contamination. After centrifugation steps, carefully remove the column from the collection tube to prevent buffer carryover. Alternatively, blot emptied collection tube with a tissue or towel. - Make sure lysate and wash buffers have passed completely through the matrix of the column. This may require centrifuging at a higher speed and/or longer time. <p>Incomplete lysis and/or cellular debris:</p> <ul style="list-style-type: none"> - Increase the volume DNA/RNA Shield™ and/or RNA Lysis Buffer (proportionally) to ensure complete lysis and homogenization. Be sure to centrifuge any cellular debris and then process the cleared lysate.
<p>Low yield</p>	<p>Sample input:</p> <ul style="list-style-type: none"> - Too much input or incomplete lysis/homogenization can cause cellular debris to clog or overload the column and result in compromised nucleic acid recovery. Use less input material and/or increase the volume DNA/RNA Shield™ and/or RNA Lysis Buffer. <p>High-protein content (blood, plasma/serum, etc.)</p> <ul style="list-style-type: none"> - Perform Proteinase K treatment to the sample prior to purification. See appropriate sample preparation protocol.
<p>DNA contamination</p>	<p>To remove DNA:</p> <ul style="list-style-type: none"> - Perform in-column DNase I treatment (page 8) or perform DNase I treatment post-purification, then re-purify the treated sample. - For future preps, increase the volume of DNA/RNA Shield™ and/or RNA Lysis Buffer to ensure complete lysis and homogenization of the sample.
<p>RNA degradation</p>	<p>To prevent RNA degradation:</p> <ul style="list-style-type: none"> - Immediately collect and lyse fresh sample into DNA/RNA Shield™ and/or RNA Lysis Buffer ensure stability. Homogenized samples can be stored frozen for later processing.

For technical assistance, please contact 1-888-882-9682 or email tech@zymoresearch.com

Notes



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Integrity of kit components is guaranteed for up to one year from date of purchase. Reagents are routinely tested on a lot-to-lot basis to ensure they provide the highest performance and reliability.

This product is for research use only and should only be used by trained professionals. It is not for use in diagnostic procedures. Some reagents included with this kit are irritants. Wear protective gloves and eye protection. Follow the safety guidelines and rules enacted by your research institution or facility.

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