ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

Direct-zol™ RNA MiniPrep

Catalog Nos. **R2050, R2051, R2052,** & **R2053**

Highlights

- Quick, spin column purification of high-quality (DNA-free) total RNA ***directly*** from TRIzol®, TRI Reagent® and all other acid-guanidinium-phenol based reagents (RNAzol®, QIAzol®, TriPure™, TriSure™, *etc.*).
- Bypasses phase separation and precipitation procedures.
- Efficient, broad range purification of small and large RNAs from cells, tissues, biological liquids, *in vitro* transcripts, *etc*.
- Ideal for viral inactivation/sample storage.

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ZYMO RESEARCH CORP.

Phone: (949) 679-1190 ▪ Toll Free: (888) 882-9682 ▪ Fax: (949) 266-9452 ▪ info@zymoresearch.com ▪ www.zymoresearch.com

Satisfaction of all Zymo Research products is guaranteed. If you should be dissatisfied with this product please call 1-888-882-9682.

Follow applicable federal, state, and local regulations for phenol waste disposal.

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.

Product Contents

Direct-zol™ RNA MiniPrep Kit Size (Preps)	**R2050** (50)	**R2051** (50)	**R2052** (200)	**R2053** (200)
TRI Reagent®	-	50 ml	-	100 ml
Direct-zol™ RNA PreWash[1] (concentrate)	40 ml	40 ml	160 ml	160 ml
RNA Wash Buffer[2] (concentrate)	12 ml	12 ml	48 ml	48 ml
DNase I Set[3] DNase I (250 U) & 10X Reaction Buffer (1 ml)	1 set	1 set	4 sets	4 sets
DNase/RNase-Free Water	6 ml	6 ml	2x 6 ml	2x 6 ml
Zymo-Spin™ IIC Columns	50	50	200	200
Collection Tubes	2x 50	2x 50	8x 50	8x 50
Instruction Manual	1	1	1	1

Storage Temperature - Store all kit components (*i.e.,* buffers, columns) at room temperature.
TRI Reagent® is provided only with catalog numbers **R2051** and **R2053**.

1 Before use, add 10 ml and 40 ml ethanol (95-100%) to the 40 ml and 160 ml **Direct-zol™ RNA PreWash** concentrate, respectively.

2 Add 48 ml 100% ethanol (52 ml 95% ethanol) to the 12 ml **RNA Wash Buffer** concentrate or 192 ml 100% ethanol (208 ml 95% ethanol) to the 48 ml **RNA Wash Buffer** concentrate before use.

3 Reconstitute the lyophilized **Dnase I** prior to use and store at -20°C (**Appendix A**, page 6).

Specifications

- **Sample Sources** – Cells from culture, solid tissue, plasma, serum, whole blood, and *in vitro* processed RNA (*e.g.,* transcription products, DNase-treated or labeled RNA) or samples stored and preserved in TRI Reagent®, TRIzol®, RNAzol®, QIAzol®, TriPure™, TriSure™ and all other *acid-guanidinium-phenol* reagents.
- **Sample Inactivation** – TRI Reagent® (provided only with R2051, R2053) inhibits RNase activity and inactivates viruses and other infectious agents.
- **RNA Size** – RNAs ≥17 nucleotides.
- **RNA Purity** – A_{260}/A_{280} >1.8, A_{260}/A_{230} >1.8. Complete removal of DNA can be accomplished with an *in-column* DNase I digestion (**Appendix A**, page 6).
- **RNA Recovery** – The RNA binding capacity of the **Zymo-Spin™ IIC Column** is ~50 µg/25 µl minimum elution volume.
- **Compatibility** – TRIzol®, RNAzol®, QIAzol®, TriPure™, TriSure™ and all other *acid-guanidinium-phenol* based solutions can be used in place of TRI Reagent®.

  Note: Compatible with samples stored in RNA*later*™ (**Appendix B**, page 6). Also, compatible with samples in TRI Reagent® that contain chloroform, 1-bromo-3-chloropropane (BCP), or 4-bromoanisole (BAN) *or* the aqueous phase of phase-separated samples (**Appendix C**, page 6).
- **RNA Storage** – RNA eluted with **DNase/RNase-Free Water** (provided) can be stored at ≤-70 ºC. The addition of RNase inhibitors is highly recommended for prolonged storage.
- **Equipment Needed** – Microcentrifuge.

Product Description

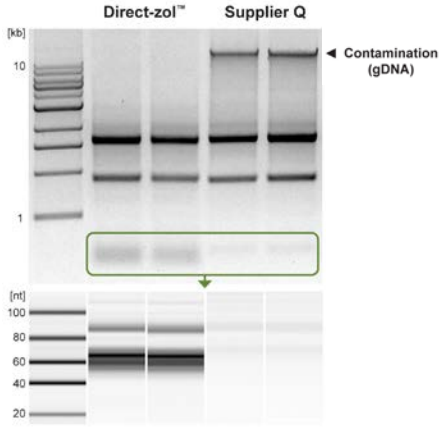
The **Direct-zol™ RNA MiniPrep** provides a streamlined method for the purification of up to 50 µg (per prep) of high-quality RNA *directly* from samples in TRI Reagent®[1]. Total RNA, including small RNAs (17-200 nt), is effectively isolated from a variety of sample sources (cells, tissues, serum, plasma, blood, biological liquids, *etc.*) using this product. The extraction method inactivates viruses and other infectious agents[2].

The procedure is easy: simply apply a sample in TRI Reagent® to the **Zymo-Spin™ IIC Column**, then spin, wash, and elute the RNA. No phase separation, precipitation, or post-purification steps are necessary. The result is broad range purification of small and large RNAs suitable for subsequent RNA-based methods including RT-PCR, transcription profiling, hybridization, *etc*.

The entire procedure typically takes about 10 minutes.

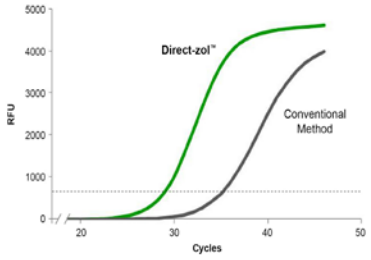
Ready-to-Use RNA

Efficient Recovery of Small & Large RNAs



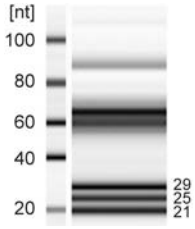
(Top) High quality broad size-range DNA-free RNA is purified from human epithelial cells using the Direct-zol™ procedure compared to a preparation from Supplier Q (1% agarose/TAE gel).
(Bottom) Small RNAs are efficiently recovered with the Direct-zol™ procedure. However, this is not the case with Supplier Q's prep (Bioanalyzer, Small RNA Chip).

Sensitive RT-PCR Detection



Viral RNA is detected with high sensitivity and improves the detection of West Nile Virus following the Direct-zol™ procedure when compared to the conventional phase-separation method. The RT-qPCR data show ΔCt = 5 (average of two independent experiments). RNA was isolated from cell-free samples inactivated using the TRI Reagent®.

Direct-zol™ Purification of miRNAs



Micro RNAs are effectively recovered from TRIzol® extracts using the Direct-zol™ procedure. miRNAs (21-29 nt) "spiked" into the extract are evidenced by a a Bioanalyzer (Small RNA Chip).

For **assistance**, please contact Zymo Research Technical Support at 1-888-882-9682 or e-mail tech@zymoresearch.com.

Note:

[1] TRIzol®, RNAzol®, QIAzol®, TriPure™, TriSure™ and all other *acid-guanidinium-phenol* reagents.

[2] For Catalog Nos. R2051, R2053 supplied with TRI Reagent®.

Catalog Nos. R2050, R2052 do not include TRI Reagent®.

Make sure RNA isolation procedure is performed in an RNase-free environment.

Buffer Preparation

- ✓ Add 10 ml and 40 ml ethanol (95-100%) to the 40 ml and 160 ml **Direct-zol™ RNA PreWash** concentrate, respectively.
- ✓ Add 48 ml 100% ethanol (52 ml 95% ethanol) to the 12 ml **RNA Wash Buffer** concentrate or 192 ml 100% ethanol (208 ml 95% ethanol) to the 48 ml **RNA Wash Buffer** concentrate.

Sample Preparation

All steps can be performed at room temperature unless specified otherwise.

The following guidelines are provided for the processing[1] of various sample types in TRI Reagent® or similar[2] prior to spin column RNA Purification.

Cell Monolayers

It is recommended to process $5x10^3$ - $5x10^6$ animal cells (per prep).

1. Lyse adherent cells[3] directly in a culture plate/dish. Add 100 µl TRI Reagent® for each cm^2 of culture surface area and mix well by pipetting. Incubate the mixture for 5 minutes at room temperature.

   Example: Add 200 µl TRI Reagent® per well of a 24-well plate (table below).

2. To remove particulates, centrifuge the mixture at 12,000 *x g* for 1 minute (or longer if necessary) and then carefully transfer the supernatant into an RNase-free tube (not provided). Proceed with **RNA Purification** (page 5).

Approximate cell number per culture area for "high-density" growth cells.

Culture Container	Well /Flask Surface Area	Cell Number
96-well plate	0.32-0.6 cm^2	$4-5x10^4$
24-well plate	2 cm^2	$1-3x10^5$
12-well plate	4 cm^2	$4-5x10^5$
6-well plate	9.5 cm^2	$0.5-1x10^6$
T25 Culture Flask	25 cm^2	$2-3x10^6$
T75 Culture Flask	75 cm^2	$0.6-1x10^7$
T175 Culture Flask	175 cm^2	$2-3x10^7$

Cell Suspensions

It is recommended to process $5x10^3$ - $5x10^6$ animal cells (per prep)[4].

1. Pellet cells by centrifugation. Carefully remove the supernatant and lyse the cell pellet directly in TRI Reagent®. Use 1 ml of the TRI Reagent® for up to 10^7 animal cells.

   Note: Alternatively, for dilute cell suspensions, add three volumes of TRI Reagent® to each volume of cell suspension.

   Mix well by vortexing and incubate the mixture for 5 minutes at room temperature.

2. To remove particulates, centrifuge the mixture at 12,000 *x g* for 1 minute (or longer if necessary) and then carefully transfer the supernatant into an RNase-free tube (not provided). Proceed with **RNA Purification** (page 5).

Notes:

[1] For detailed processing information, refer to the TRI Reagent® product manual (or manufacturer's instructions for the reagent used).

[2] TRIzol®, RNAzol®, QIAzol®, TriPure™, TriSure™ and all other *acid-guanidinium-phenol* reagents.

[3] Although cell types and culture conditions may vary, the procedure is compatible with high-density growth cells (e.g., HeLa cells) as well as low-density ones (e.g., neuronal cells).

[4] For homogenization of tough-to-lyse microbial samples, use **ZR BashingBead™ Lysis Tubes** (S6002-50) with disrupters/ pulverizers fitted with a 2 ml tube holder assembly.

Biological Liquids

Up to 100 µl of biological liquid per prep (*e.g.,* blood, serum, plasma, semen, CSF, buffy coat, body fluids) can be processed without having to reload the spin column.

1. Add three volumes of TRI Reagent® to each volume of liquid sample. Mix well by vortexing and incubate the mixture for 5 minutes at room temperature.

   Note: When sampling **whole blood** or **plasma** supplement each 100 µl sample with 10 µl of 5 N acetic acid.

2. To remove particulates, centrifuge the mixture at 12,000 *x g* for 1 minute (or longer if necessary) and then carefully transfer the supernatant into an RNase-free tube (not provided). Proceed with **RNA Purification** (page 5).

Tissue

An equivalent of up to 50 mg tissue (per prep) can be sampled with this kit. Larger samples can exceed the RNA binding capacity of the spin column.

1. Add at least 500 µl TRI Reagent® per 50 mg tissue. Homogenize using **ZR BashingBead™ Lysis Tubes**[1], **Squisher™**[2], a glass-Teflon, Polytron, or similar homogenizer.

   Note: Sample should not exceed 10% of the TRI Reagent® volume used for homogenization.

2. To remove particulates, centrifuge the mixture at 12,000 *x g* for 1 minute (or longer if necessary) and then carefully transfer the supernatant into an RNase-free tube (not provided). Proceed with **RNA Purification** (page 5).

In vitro Reactions

For cleanup of enzymatic reactions (*e.g., in vitro* transcription products, DNase-treated or labeled RNA), add three volumes TRI Reagent® to each volume of sample and mix by vortexing. Proceed with the **RNA Purification** (page 5).

Example: Add 300 µl TRI Reagent® to a 100 µl reaction.

For Samples already homogenized in TRI Reagent®

Remove particulates from **cell** and **tissue** sample homogenates in TRI Reagent® by centrifugation at 12,000 *x g* for 1 minute (or longer if necessary) and then carefully transfer the supernatant into an RNase-free tube (not provided). Proceed with **RNA Purification** (page 5).

Notes:

[1] For homogenization of tough-to-lyse small tissue samples, use **ZR BashingBead™ Lysis Tubes** (S6003-50) with disrupters/ pulverizers fitted with a 2 ml tube holder assembly.

[2] **Squisher™** homogenizers (H1001, H1002, H1004) are available from Zymo Research.

RNA Purification

All centrifugation steps should be performed at 10,000-16,000 x *g.*

1. Add one volume ethanol (95-100%) directly to one volume sample homogenate (1:1) in TRI Reagent® or similar[1]. Mix well by vortexing.

2. Load the mixture into a **Zymo-Spin™ IIC Column**[2] in a **Collection Tube** and centrifuge for 1 minute. Transfer the column into a new **Collection Tube** and discard the **Collection Tube** containing the flow-through.

   Note: At this point, RNA samples can be *in-column* DNase treated (**Appendix A**, page 6).

3. Add 400 µl **Direct-zol™ RNA PreWash**[3] to the column and centrifuge for 1 minute. Discard the flow-through. *Repeat this step.*

   Add 700 µl **RNA Wash Buffer**[3] to the column and centrifuge for 1 minute. Discard the flow-through. To ensure complete removal of the wash buffer, centrifuge the column for an additional 2 minutes in an emptied **Collection Tube**. Transfer the column carefully into an RNase-free tube (not provided).

4. Add ≥25 µl of **DNase/RNase-Free Water**[4] directly to the column matrix and centrifuge at max speed for 1 minute.

   The eluted RNA can be used immediately or stored at ≤-70°C.

Notes:

[1] TRIzol®, RNAzol®, QIAzol®, TriPure™, TriSure™ and all other *acid-guanidinium-phenol* reagents.

[2] To process samples >700 ul, reload the column and repeat Step 2 or use a vacuum manifold.

[3] Before use, add ethanol to the buffer concentrate (**Buffer Preparation**, page 3).

[4] For maximum recovery, increase the elution volume (≥50 µl) and/or repeat the elution.

Appendix A: In-Column DNase I digestion

All centrifugation steps should be performed at 10,000-16,000 x *g*.

During RNA Purification (step 2, page 5):

1. Add 400 µl **RNA Wash Buffer** to the **Zymo-Spin™ IIC Column** in a **Collection Tube** and centrifuge for 1 minute. Discard the flow-through.

   Prepare DNase I cocktail:

		Individual Reaction
Example:	**DNase I** (250 U/vial; lyophilized)	5 U*
	10X DNase I Reaction Buffer	8 µl
	DNase/RNase-Free Water	3 µl
	RNA Wash Buffer[1]	64 µl

   ***** Add 275 µl DNase/RNase-Free Water per vial to reconstitute the lyophilized DNase I (E1009) at 1 U/µl.

2. Mix by gentle inversion in an RNase-free tube (not provided). Add 80 µl of the DNase I cocktail directly to the matrix of the **Zymo-Spin™ IIC Column**. Keep the column in an emptied **Collection Tube**. If needed, store any leftover cocktail at -20°C.
3. Incubate the column at 25-37ºC for 15 minutes[2], then centrifuge for 30 seconds.

   Continue with RNA Purification (step 3, page 5).

Notes:

[1] When adjusting volume and composition, make sure the **RNA Wash Buffer** in the **DNase I cocktail** remains at 80% (v/v).

Unit definition - one unit increases the absorbance of a high molecular weight DNA solution at a rate of 0.001 A_{260} units/min/ml of reaction mixture at 25°C.

[2] The optimal incubation time can vary.

Appendix B: RNA extraction from samples stored in RNA*later*™

Cells

Pellet cells[3] at up to 5,000 x *g* and remove the RNA*later*™ (the supernatant) prior to RNA extraction. Then immediately lyse the cell pellet in TRI Reagent®[4,5] (Sample Preparation, Cell Suspensions, page 3).

> **Note:** To extract RNA from cells without reagent removal, use 10 volumes of TRI Reagent® per sample volume. Proceed to phase separation and process the aqueous phase. Simply transfer the aqueous phase containing RNA into an RNase-free tube (not provided). Then, proceed to RNA Purification (page 5).

Tissue

Remove tissue from RNA*later*™ using forceps. Eliminate any excess reagent or crystals that may have formed and proceed immediately with extraction in TRI Reagent® or similar[4] (Sample Preparation, Tissue, page 4).

[3] Different cells may react differently to centrifugation forces and it is recommended to test the pelleting procedure with non-valuable samples first. Diluting RNA*later*™ by 50% with cold PBS reduces solution density allowing for lower forces during cell pelleting.

[4] TRIzol®, RNAzol®, QIAzol®, TriPure™, TriSure™ and all other *acid-guanidinium-phenol* reagents.

[5] For detailed processing information, refer to the TRI-Reagent® product manual (or manufacturer's instructions for the reagent used).

Appendix C: Aqueous phase of phase-separated samples

For samples that have already been phase separated in TRI Reagent® or similar[4], simply transfer the aqueous phase containing RNA into an RNase-free tube (not provided). Then, proceed to RNA Purification (page 5).

Ordering Information

Product Description	Catalog No.	Kit Size
Direct-zol™ RNA MiniPrep (TRI Reagent® not included)	R2050 R2052	50 preps. 200 preps.
Direct-zol™ RNA MiniPrep (supplied with TRI Reagent®)	R2051 R2053	50 preps. 200 preps.

Individual Kit Components	Catalog No.	Amount
TRI Reagent®	R2050-1-50 R2050-1-100	50 ml 100 ml
Direct-zol™ RNA PreWash (concentrate)	R2050-2-40 R2050-2-160	40 ml 160 ml
RNA Wash Buffer (concentrate)	R1003-3-6 R1003-3-12 R1003-3-24 R1003-3-48	6 ml 12 ml 24 ml 48 ml
Zymo-Spin™ IIC Columns	C1011-50 C1011-250	50 250
Collection Tubes	C1001-50 C1001-500 C1001-1000	50 500 1000
DNase/RNase-Free Water	W1001-1 W1001-6 W1001-10	1 ml 6 ml 10 ml
DNase I (lyophilized) (250 U supplied with 10X Reaction Buffer)	E1009	1 set

Related Products

Product	Description	Prep/Format	Catalog
RNA Clean-Up			
RNA Clean & Concentrator™-5	Cleanup and concentration of modified, labeled, impure, diluted, DNase treated RNA (≥ 17nt) and purification of RNA from aqueous phase of organic extracts. *Note: DNA-free RNA Kit™ includes DNase I*	50/column 200/column	R1015 R1016
RNA Clean & Concentrator™-25		50/column 100/column	R1017 R1018
RNA Clean & Concentrator™-100		25/column	R1019
ZR-96 RNA Clean & Concentrator™		2x 96/plate	R1080
DNA-Free RNA Kit™		50/column 200/column	R1013 R1014
Oligo Clean & Concentrator™	Cleanup and concentration of RNA and/or DNA oligos. Good for clean-up of miRNAs and siRNAs.	50/column 200/column	D4060 D4061
ZR-96 Oligo Clean & Concentrator™		2x 96/plate 4x 96/plate	D4062 D4063
ssDNA/RNA Clean & Concentrator™	Separation of short ssRNA and ssDNA (up to 200 nt) from double stranded species.	20/column 50/column	D7010 D7011
Zymoclean™ Gel RNA Recovery Kit	Recovery of RNA from agarose gels.	50/column	R1011
ZR small-RNA™ PAGE Recovery Kit	Small RNA (> 17nt) from polyacrylamide gels.	20/column	R1070
***OneStep™* PCR Inhibitor Removal Kit**	Removal of polyphenolics, humic/fulvic acids, tannins, melanin etc. from RNA.	50/column	D6030
***OneStep™* -96 PCR Inhibitor Removal Kit**		2x 96/plate	D6035
RNA from Samples in TRI Reagent (Small RNA Recovery)			
Direct-zol™ RNA MiniPrep	RNA (>17 nt) from TRI Reagent®, TRIzol®, and all other acid-guanidinium-phenol based reagents without phase separation.	50/column 200/column	R2050 R2052
Direct-zol™ RNA MiniPrep w/ TRI Reagent		50/column 200/column	R2051 R2053
Direct-zol™-96 RNA		2x 96/plate 4x 96/plate	R2054 R2056
Direct-zol™-96 RNA w/ TRI Reagent		2x 96/plate 4x 96/plate	R2055 R2057
Direct-zol™-96 MagBead RNA	RNA (>17 nt) from TRI Reagent®, TRIzol®, and all other acid-guanidinium-phenol based reagents without phase separation. These kits are in a magnetic bead format that is adaptable for high-throughput and automated protocols.	2x 96/plate 4x 96/plate 8x 96/plate	R2100 R2102 R2104
Direct-zol™-96 MagBead RNA w/ TRI Reagent		2x 96/plate 4x 96/plate 8x 96/plate	R2101 R2103 R2105
RNA from Cells			
***Quick-RNA™* MicroPrep**	Total RNA from cells.	50/column 200/column	R1050 R1051
***Quick-RNA™* MiniPrep**		50/column 200/column	R1054 R1055
***Quick-RNA™* MidiPrep**		25/column	R1056
ZR-96 *Quick-RNA™*		2x 96/plate 4x 96/plate	R1052 R1053
RNA from Tissue			
ZR RNA MicroPrep™	Total RNA (>17 nt) from fresh/frozen tissue.	50/column 200/column	R1060 R1061
ZR RNA MiniPrep™		50/column 200/column	R1064 R1065
ZR-*Duet*™ DNA/RNA MiniPrep	Parallel purification of DNA/RNA from cells.	50/column	D7001
Pinpoint™ Slide RNA Isolation System Kit I	RNA from fresh/frozen tissue sections.	50/column	R1003
Pinpoint™ Slide RNA Isolation System Kit II	RNA from paraffin-embedded (FFPE) tissue.	50/column	R1007
RNA from Biological Liquids			
ZR Viral RNA Kit™	RNA (DNA) from body fluids (plasma, serum, CSF, urine).	50/column 200/column	R1034 R1035
ZR-96 Viral RNA Kit™		2x 96/plate 4x 96/plate	R1040 R1041
ZR Viral DNA/RNA Kit™		25/column 100/column	D7020 D7021
ZR Whole-Blood RNA MiniPrep™	RNA from whole blood or partitioned blood.	50/column 100/column	R1020 R1021
ZR Urine RNA Isolation Kit™	Cellular and endosomal RNA from urine.	20/column 50/column	R1038 R1039
RNA from Tough-to-Lyse Samples			
ZR Fungal/Bacterial RNA MicroPrep™	RNA from bacteria, yeast, fungi; BashingBead™ lysis.	50/column	R2010
ZR Fungal/Bacterial RNA MiniPrep™		50/column	R2014
ZR Plant RNA MiniPrep™	RNA from leaves, stems, buds, flowers, fruits, seeds, etc; BashingBead™ lysis, RT/PCR inhibitor removal.	50/column	R2024
ZR Tissue & Insect RNA MicroPrep™	RNA from insect, arthropod specimen and small tissue samples; BashingBead™ lysis.	50/column	R2030
ZR Soil/Fecal RNA MicroPrep™	RNA from soil, sludge, sediment, feces.	50/column	R2040
YeaStar RNA Kit™	RNA from yeast strains susceptible to Zymolyase.	50/column	R1002
RNA Sample Preservation and Storage			
RNA Shield™	Cells, biological liquid, tissue storage and RNA purification.	50 ml 250 ml	R1100-50 R1100-250
RNA Shield™ Purification Kit (RNA Shield™ reagent included)		50/column	R1100
RNA Shield™ Purification Kit (RNA Shield™ reagent is <u>not</u> included)		50/column	R1101
Enzymes and Markers			
DNase I w/ 10X Reaction Buffer	100 µl solution	100 U	E1007
	Lyophilized	250 U	E1009
ZR small-RNA™ Ladder	ssRNA (17, 21, 25, 29 nt)	10 µg	R1090