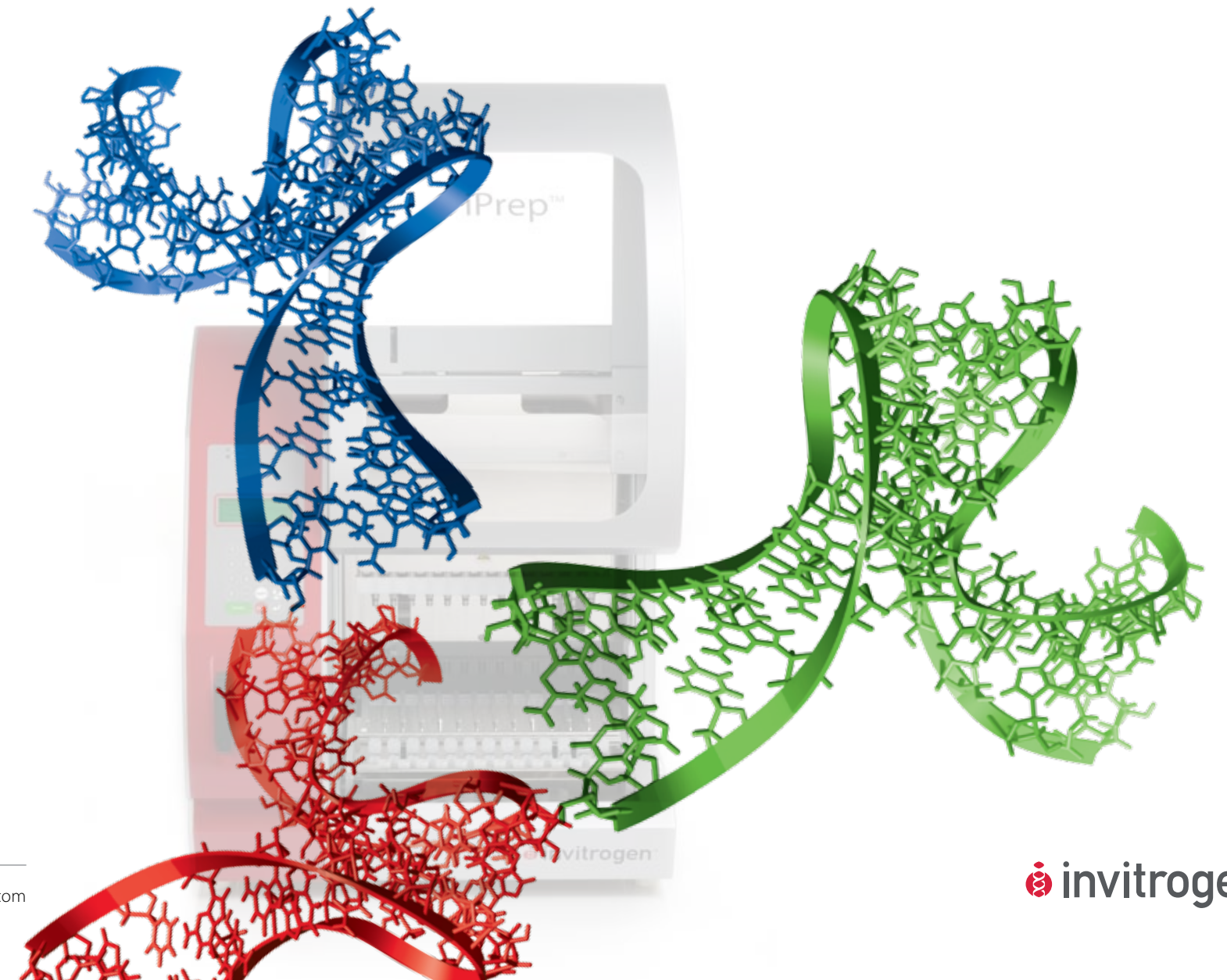


Ordering information

Product	Quantity	Cat. no.
iPrep™ PureLink™ Total RNA Kit	52 preps	IS-10006
iPrep™ Trizol® Plus RNA Kit	52 preps	IS-10007
iPrep™ RNA Card (includes software for iPrep™ Trizol® Plus RNA and iPrep™ PureLink™ Total RNA protocols)	1 card	IS-10014
Related products		
iPrep™ Purification Instrument	1 unit	IS-10000
iPrep™ ChargeSwitch® Forensic Kit	52 preps	IS-10002
iPrep™ ChargeSwitch® Buccal Cell Kit	52 preps	IS-10003
iPrep™ ChargeSwitch® gDNA Tissue Kit	52 preps	IS-10004
iPrep™ PureLink™ gDNA Blood Kit	52 preps	IS-10005
iPrep™ Forensic Card (includes buccal protocol)	1 card	IS-10011
iPrep™ Card: gDNA Blood	1 card	IS-10012
iPrep™ Card: gDNA Tissue	1 card	IS-10013

Reliable RNA sample preparation
from tissue, cells, and blood

iPrep™ TRizol® Plus RNA Kit and iPrep™ PureLink™ Total RNA Kit





Reliable RNA sample preparation from tissue, cells, and blood

iPrep™ TRizol® Plus RNA and iPrep™ PureLink™ Total RNA Kits

- Highly pure and intact RNA
- High yields from even the smallest samples
- Full RNA representation

Most gene expression profiling studies, such as RT-PCR, microarray, northern analysis, and cDNA library construction, begin with RNA purification. Variability in this purification step is a main source of

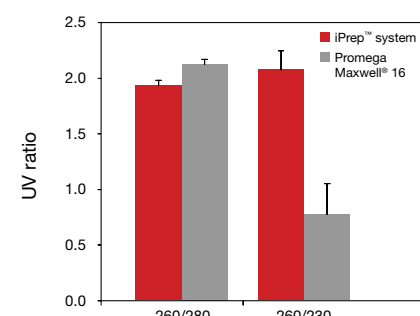


Figure 1—Purity comparison of RNA obtained by automated extraction. RNA was purified from 10 mg of various mouse tissue (n = 11) samples using the iPrep™ PureLink™ Total RNA Kit on the iPrep™ Purification Instrument and also using the Promega Maxwell® 16 kit (n = 10). UV ratios were measured using a NanoDrop® ND-1000 spectrophotometer.

Table 1—Overview of the iPrep™ RNA purification kits.

iPrep™ kit	Advantage	Sample preparation	Run time	Highest yield	Highest purity	Highest reproducibility	Starting material					Complete RNA representation
							Tissue	Fatty tissue	Cells	WBC	Plant tissue	
iPrep™ TRizol® Plus RNA Kit	Higher yields from larger samples	Tissue disruption & organic phase separation step (25 min)	30 min	+++	+++	+++	up to 50 mg sample	up to 50 mg sample	up to 1 × 10 ⁷ cells	NR	up to 100 mg sample	++
iPrep™ PureLink™ Total RNA Kit	Less hands-on time; sensitive isolation from small samples	Tissue disruption & addition of lysis buffer (10 min)	45 min	++	++	+++	up to 10 mg sample	NR	up to 1 × 10 ⁶ cells	up to 1 ml of blood	NR	++

NR = Kit not recommended for this sample type.

high statistical variance in downstream applications. To bring greater purity, yield, and reproducibility to your RNA purification needs, we have developed three protocols—for cultured cells, tissue, and whole blood samples—to achieve total RNA purification using iPrep™ RNA purification kits (Table 1) and the iPrep™ Purification Instrument.

Yield from various sample types

Using Dynabeads® magnetic separation technology, iPrep™ RNA kits purify large amounts of highly pure RNA sufficient for multiple uses with the full range of downstream applications. RNA content varies widely, depending on the quality of the starting sample. Table 2 outlines typical RNA yields from various samples. The observed A_{260}/A_{280} ratios are within the optimum 1.9–2.1 range.

Purity and integrity of RNA

While the A_{260}/A_{280} ratio is useful for assessing protein contamination, the A_{260}/A_{230} ratio is a useful indicator of other contaminants—low A_{260}/A_{230} ratios may indicate the presence of organics or salts in the

Table 2—Yield and purity of RNA purified using the iPrep™ TRizol® Plus RNA Kit or the iPrep™ PureLink™ Total RNA Kit.

Sample	iPrep™ TRizol® Plus RNA Kit		iPrep™ PureLink™ Total RNA Kit	
	Mean total RNA yield, in µg (range)	A_{260}/A_{280} ratio	Mean total RNA yield, in µg (range)	A_{260}/A_{280} ratio
293 cells (1 × 10 ⁷ /1 × 10 ⁶)	52.2 (50.1–55.5)	2.0	10.9 (10.1–11.6)	2.0
Liver (10 mg)	44.2 (43.3–46.3)	2.1	40.8 (35.9–44.5)	2.1
Kidney (10 mg)	9.2 (7.2–12.3)	1.9	7.0 (5.8–8.7)	2.1
Heart (10 mg)	4.8 (3.7–6.4)	1.9	1.9 (1.1–2.3)	2.0
Spleen (10 mg)	33.5 (27.7–37.6)	1.9	(kit not recommended for this sample type)	
<i>E. coli</i> (0.5 ml of culture, OD ₆₀₀ = 1)	80.3 (79.0–81.7)	1.9	64.8 (58.0–68.5)	2.0
Tomato leaf (100 mg)	23.9 (23.1–25.4)	2.0	(kit not recommended for this sample type)	

Yield and purity are given as mean values, with the range of values in parentheses. RNA was purified using the specified kit and the iPrep™ Purification Instrument. RNA was eluted in 100 µl elution buffer; yield was determined using the Quant-iT™ RNA Assay (Cat. no. Q-33140). The UV absorbance ratios were measured using a NanoDrop® ND-1000 spectrophotometer.

eluted RNA that can interfere with various PCR methods. RNA purified using the iPrep™ PureLink™ Total RNA Kit consistently shows A_{260}/A_{230} > 2.0, indicating the absence of such contaminants (Figure 1).

Significant RNA digestion can occur during the RNA purification process; however, samples purified using the iPrep™ system show a consistently high RNA integrity number (RIN) for each sample type, in contrast to the performance of some competitors, especially on difficult tissue such as spleen (Figures 2 and 3).

Full RNA transcript representation

The iPrep™ RNA kits on the iPrep™ instrument successfully purify total RNA content, including small molecular weight RNAs such as tRNA, 5S RNA, and other regulatory RNA. This is in contrast to some competing kits, which show a notable absence of small molecular weight RNA species (Figure 4). The resulting RNA shows highly

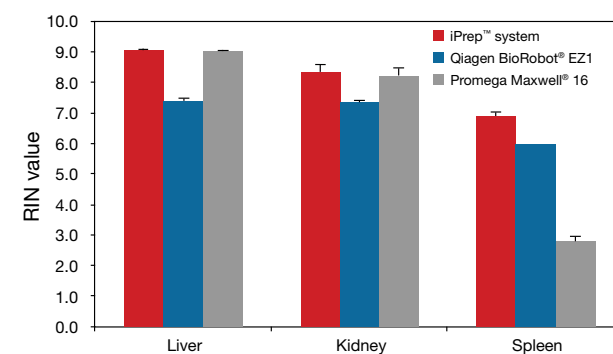


Figure 2—Integrity comparison of RNA obtained by automated extraction. RNA was purified from 10 mg of mouse liver, kidney, and spleen tissue using the iPrep™ TRizol® Plus RNA Kit on the iPrep™ Purification Instrument versus the Qiagen BioRobot® EZ1 and Promega Maxwell® 16 systems.

consistent performance in RT-PCR and qRT-PCR reactions, which are highly sensitive to the inhibitory effects of contaminants. Variation in downstream processing is minimal, giving you more consistent results (Figure 5).

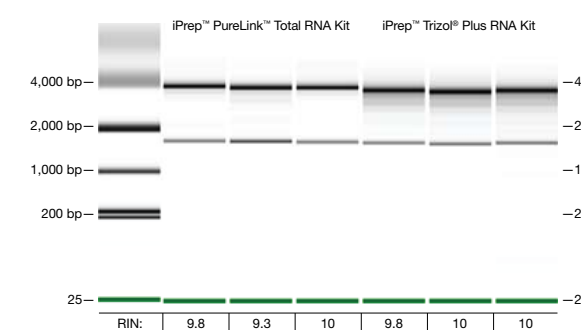


Figure 3—Integrity of RNA obtained by automated extraction. RNA was purified from 10 mg of mouse liver using the iPrep™ PureLink™ Total RNA Kit and the iPrep™ TRizol® Plus RNA Kit on the iPrep™ Purification Instrument. Purified RNA was analysed on the Agilent Bioanalyser, and the RIN numbers were calculated. The gel image shows triplicate data illustrating high RNA integrity and consistently high RIN numbers from both kits.

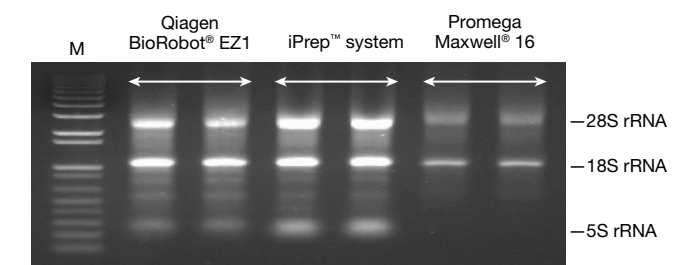


Figure 4—Ribosomal RNA isolated using the iPrep™ system. RNA purified from 10 mg of mouse liver tissue using the iPrep™ TRizol® Plus RNA Kit on the iPrep™ Purification Instrument was electrophoresed on an E-Gel® 12 1% agarose gel (nondenaturing). Lane M is a 1 Kb Plus Ladder. Ribosomal RNA bands are clearly visible, indicating efficient extraction of total RNA, whereas the RNA isolated using competing systems shows absence of lower molecular weight RNA.

Reproducible, contaminant-free RNA purification

Using the iPrep™ instrument, the iPrep™ RNA kits provide RNA yields that are linear over a wide range of sample input material and that directly correlate with the amount of RNA present in the sample (Figure 6). Due to precise liquid handling and chemistry, purification using the iPrep™ system yields highly reproducible results (CV<10%), so you can be sure that detected differences in expression levels do not reflect variability in the purification method (Table 3). Prefilled, sealed cartridges used in the iPrep™ system ensure that no cross-contamination occurs between samples (Figure 7).

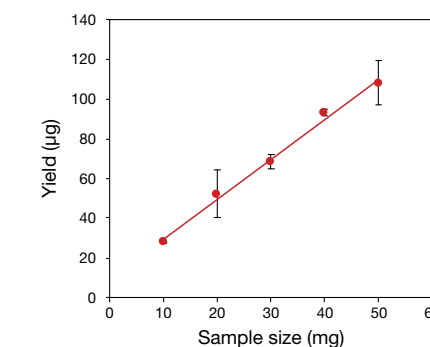


Figure 6—Linear RNA extraction achieved with the iPrep™ system. RNA was purified from 10, 20, 30, 40, and 50 mg of mouse liver tissue using the iPrep™ TRizol® Plus RNA Kit and the iPrep™ Purification Instrument. RNA was eluted in 100 µl elution buffer; yield was determined using the Quant-iT™ RNA Assay. There is a linear correlation ($r^2 = 0.99$) between the quantity of starting tissue sample and the yield of purified RNA.

Extracting RNA from blood

The iPrep™ PureLink™ Total RNA Kit can be used in conjunction with the iPrep™ Purification Instrument to purify RNA from blood samples. RNA can be purified from up to 1 ml of a white blood cell (WBC) preparation; the purified RNA has been shown to successfully amplify in downstream qRT-PCR analysis (Figure 8).

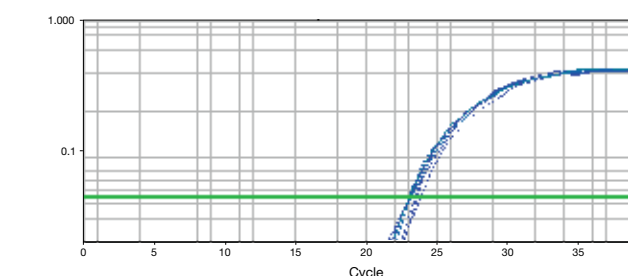


Figure 5—RNA extracted from 10 mg of liver tissue using the iPrep™ PureLink™ Total RNA Kit on the iPrep™ Purification Instrument. Purified RNA was used as a template for a qRT-PCR reaction using primers for GAPDH. The method shows strong amplification of the target with C_t values of 22.8 ± 0.3 (n = 4, iPrep™ PureLink™ Total RNA Kit).

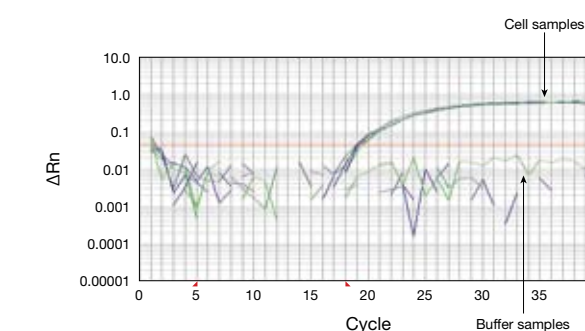


Figure 7—Cross-contamination-free RNA purification. The iPrep™ PureLink™ Total RNA Kit with optional DNase digestion was used along with the iPrep™ Purification Instrument on a series of positive (1 × 10⁶ 293 cells) and negative (600 µl lysis buffer) samples. After extraction, the RNA was used in a qRT-PCR reaction with primers for β-actin. There was no amplification in any of the negative samples, indicating an absence of cross-contamination.

Table 3—Highly reproducible extraction of RNA from 5 mg of mouse liver.

Statistical parameter	Run 1	Run 2
Minimum	14.8 µg	15.5 µg
Maximum	18.4 µg	19.4 µg
Mean	16.7 µg	17.5 µg
Standard deviation	1.4 µg	1.2 µg
CV	8.3%	7.1%
Combined CV	7.9%	

A pooled lysate was processed on 12 lanes over two separate runs (total 24 samples processed) using the iPrep™ PureLink™ Total RNA Kit and the iPrep™ Purification Instrument. The statistical data for each run is summarized. The combined CV for two runs with the same sample was 7.9%, demonstrating highly reproducible chemistry and liquid handling.

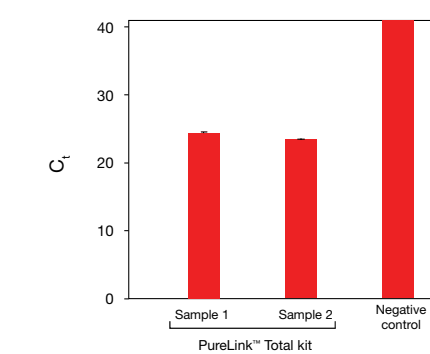


Figure 8—Downstream qRT-PCR of RNA purified from whole blood on the iPrep™ Purification Instrument using the iPrep™ PureLink™ Total RNA Kit. Purified RNA was used as template for cDNA synthesis followed by PCR amplification using primers for GAPDH.