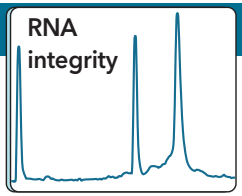
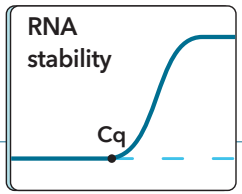
ACTIVblue
QUALITY IS IN OUR RNA

DRD Blood

is an innovative whole blood RNA stabilization solution for venous blood (1 mL), capillary blood (50 µL), or transfer from EDTA/citrate tube (50 µL). It has superior stability performance, is compatible with various easy-to-use and scalable RNA purification methods, and comes with a fair price.

Performance summary

RNA integrity is defined as a high and stable RIN value denoting intact 18S and 28S ribosomal RNA. Molecular RNA stability is defined as an RT-qPCR Cq value that does not change over time.

	4 °C	25 °C
	14 days	1 day
	30 days	3 days

Excellent freeze-thaw stability

Venous blood (1 mL) from a healthy human donor was drawn in a DRD Blood Tube and frozen at -20 °C. Over a three-week period, the blood was thawed to room temperature and frozen again, for up to 5 times. The electropherogram shows intact 18S and 28S ribosomal RNA bands with good RNA integrity (RIN) values.

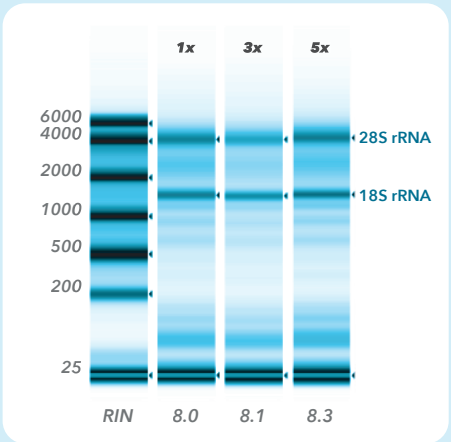


Figure 1: *TapeStation electropherogram of total RNA purified from DRD Blood frozen at -20 °C and thawed up to 5 times over three weeks.*

RNA remains intact in the fridge for 2 weeks

Venous blood (1 mL) from a healthy human donor was drawn in a DRD Blood Tube and stored at 4 °C for up to 2 weeks. At time point 0 and days 1, 3, 7, and 14, an aliquot of 200 µL stabilized blood was extracted (miRNeasy Micro). The electropherogram shows intact 18S and 28S ribosomal RNA bands with good RNA integrity (RIN) values.

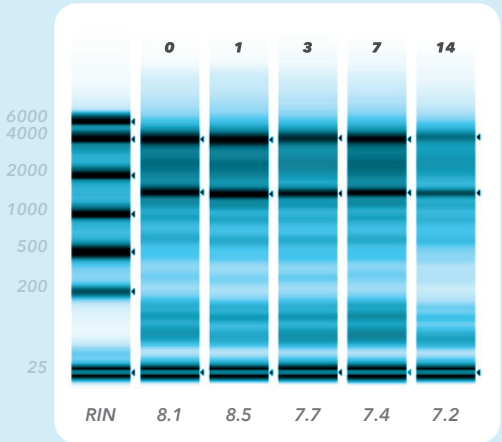


Figure 2: *TapeStation electropherogram of total RNA purified from DRD Blood stored at 4 °C for up to 14 days.*

RT-qPCR stability of selected genes at 37 °C for 1 day

An aliquot of DRD Blood was processed for RNA extraction immediately after the draw, and another was stored at 37 °C for 24 hrs, followed by RNA extraction (miRNeasy Micro). RT-qPCR analysis of 7 human genes (red blood cell marker HBB, white blood cell marker PTPRC (CD45), instability markers IL1B and FOS, and 3 reference genes (18S, GAPDH, RSP18)) demonstrated excellent stability (maximum Cq value difference of 1.5; mean difference of 0.8 cycles).

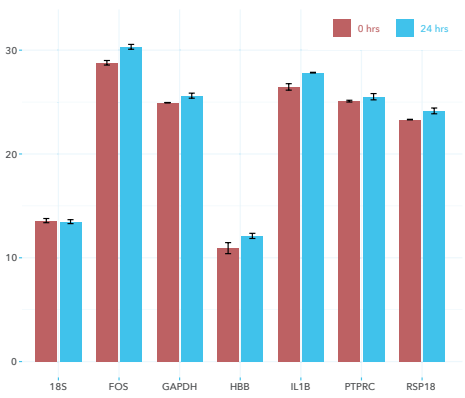
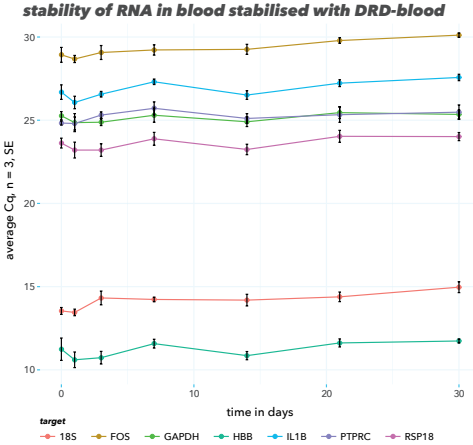


Figure 3: *RT-qPCR analysis of 7 human genes (18S rRNA, FOS, GAPDH, HBB, IL1B, PTPRC (CD45), and RSP18) in DRD Blood from healthy donors (n=3). Average Cq values with standard error of the mean (SEM) are show at time point 0 and 24 hrs after storage at 37 °C.*

RT-qPCR stability of selected genes at 4 °C for 30 days



An aliquot of DRD Blood was processed for RNA extraction immediately after the draw, and another was stored at 4 °C for 1, 3, 7, 14, 21, and 30 days, followed by RNA extraction (miRNeasy Micro).

RT-qPCR analysis of 7 human genes (red blood cell marker HBB, white blood cell marker PTPRC (CD45), instability markers IL1B and FOS, and 3 reference genes (18S, GAPDH, RSP18)) demonstrated excellent stability (maximum Cq value difference of 1.5; mean difference of 1.1 cycles).

When target levels are normalized against the reference genes, the maximum difference is 0.6 cycles over the 30 days period.

Validated RNA extraction methods

The following RNA extraction methods were successfully validated for DRD Blood. These methods use 200 - 400 µL of stabilized DRD Blood as input in the extraction (equivalent to 50 - 100 µL neat blood). The yield is 0.6-1 µg of RNA per 100 µL of neat blood.

kit	supplier	catalog #	principle
miRNeasy Micro	Qiagen	217084	spin column
MagMAX mirVana Total RNA	ThermoFisher	A27828	magnetic beads
NucleoMag RNA Blood	Macherey - Nagel	744352	magnetic beads
VAMNE Magnetic Cell/Tissue Total RNA Kit	Vazyme	RMA101-C2	magnetic beads

Table 1: *Validated RNA extraction methods for DRD Blood*

Detailed RNA extraction protocols are available on the DRD Blood webpage.

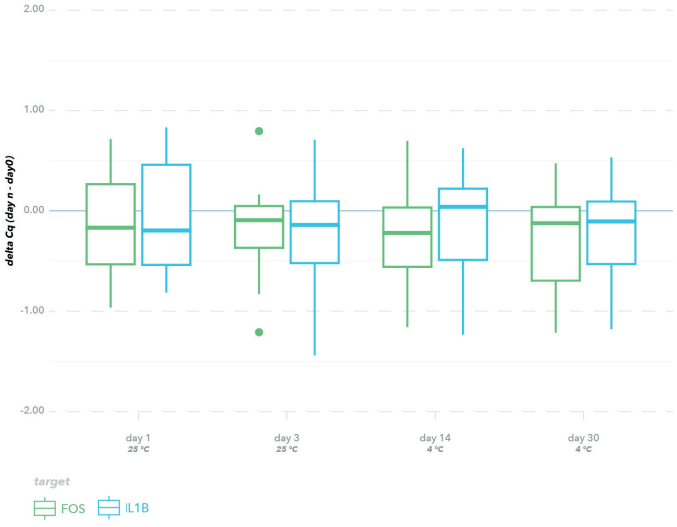
Stable RNA eluate concentration over time

DRD Blood tubes provide consistent RNA yields when stored at 4 °C or 25 °C for up to 30 days. RNA was extracted from 200 µL stabilized blood from 3 different donors using miRNeasy Micro and eluted in 20 µL. RNA concentrations were determined using a spectrophotometer.

	day 0	day 1	day 3	day 7	day 14	day 21	day 30
4 °C	21.3 (0.9)	26.1 (1.9)	35.2 (3.5)	26 (2.1)	24.3 (2.5)	19.6 (1.4)	29.4 (0.0)
25 °C	23.1 (3.2)	25.8 (1.1)	31.3 (3.8)	24.3 (0.6)	/	/	/

Table 2: *Average RNA concentration (ng/µL) of 3 healthy donors (+/- SEM)*

Expression stability of FOS and IL1B marker transcripts



FOS and IL1B are well-known transcripts in human blood that quickly change their abundance upon stress or perturbation. This figure demonstrates that their expression levels remain stable in DRD Blood collection tubes for up to 30 days at 4 °C and 3 days at 25 °C. This is an exceptional RNA stability result, considering raw data with 5 levels of potential variability, i.e. donor (n=3), DRD Blood production lot number (n=3), RNA extraction (n=1), RT-qPCR (n=2), and experiment (n=2). Each box plot represents 18 independent data points. The median delta-Cq is 0.33, the 95% delta-Cq is 1.08 (comparing a later time point with time point 0).

InActiv Blue bv
Industriepark Oost 2A
B-8730 Beernem
Belgium

VAT BE0758 884 250
+32 (0)50 79 18 07
info@inactivblue.com
www.inactivblue.com