

Tech Note NucleoSpin® RNA – Protocol recommendations for RNA isolation from insects



Introduction

Insects constitute the largest and most diverse group of animals on earth. Isolating RNA from insects is becoming increasingly important in molecular entomology, especially when studying agriculturally or epidemiologically important insects.

For molecular biology applications such as insect gene expression studies the RNA purification method needs to provide high-quality and quantity RNA while avoiding RNA degradation and contamination.

While RNA isolation from pure insect cells is generally easy due to the absence of cell walls, the physical and biochemical structure of difficult-to-lyse chitin shells makes RNA isolation from whole insects more challenging. In this Tech-Note we describe a robust method allowing RNA isolation from whole insects using the NucleoSpin® RNA kit. The method combines the efficient lysis- and washing buffer chemistry of the NucleoSpin® RNA kit with mechanical bead beating using 5 mm steel beads (MN Bead Tubes Type G).

Specifications

NucleoSpin RNA	
Technology	Silica membrane technology, Mini prep
Sample material	Insect sample, fresh, frozen (-70°C), stabilized in NucleoProtect®
Elution volume	30–120 µL
Homogenization	Bead beating, MN Bead Tubes Type G
Typc. downstream application	RNA-Seq, RT-PCR, RT-qPCR
Preparation time	35 min/6 preps



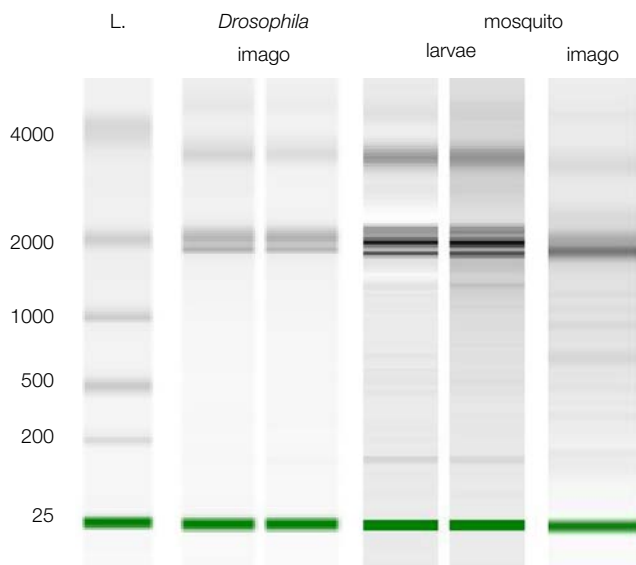
Methods

The following protocol was verified with *Drosophila* flies (imago) and mosquitos (larvae and imago).

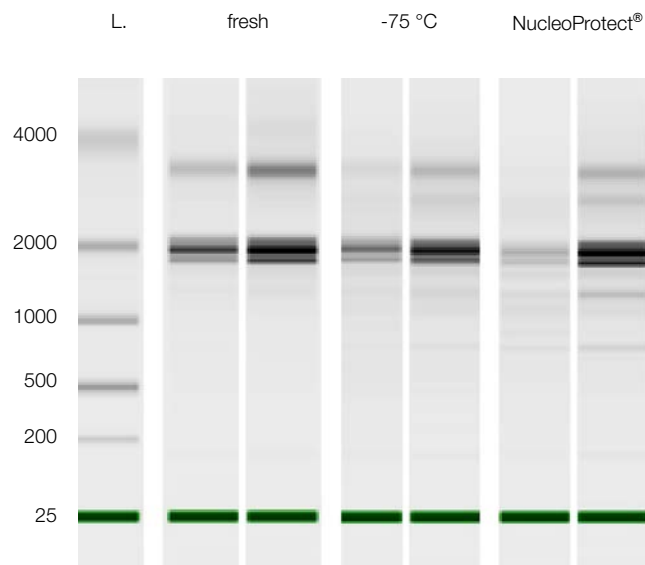
Procedure		
1	Collect samples	Use fresh, shock-frozen (-70°C.) or NucleoProtect* stabilized samples (approx. 1–10 mg of insect). * Make sure to remove excess NucleoProtect® RNA solution from the sample before starting the RNA isolation procedure.
2	Resuspension	Add 350 µL Lysis Buffer RA1 without β-mercaptoethanol or TCEP to a MN Bead Tube Type G and add the insect sample.
3	Homogenization and lysis	Perform bead beating using a retsch mill (30 Hz, 1–3 min) or MN Bead Tube Holder (full speed, 10 min).
4	Sedimentation of beads	Remove the steel balls from the bead tube (e.g. with a magnet), recover the lysate and apply it onto the NucleoSpin Filter (violet ring) according to step 3 of user manual section 5.1.

Application data

A



B



C

RNA isolation using MN Bead Tubes Type G

	<i>Drosophila</i>		mosquito
	adult	larvae	imago
Input [mg]	1–3	3–7	0.7–2
Yield [µg]	4–20	4–12	3
OD 260/280	2.3	2.2	2.2
OD 260/230	2.9	2.7	2.3
RIN	8.0	7–7.5	6.0

Insect RNA isolation using NucleoSpin® RNA with MN Bead Tube Type G

The insect RNA was isolated as described above using the NucleoSpin® RNA kit in combination with mechanical lysis using MN Bead Tubes Type G (MN Bead Tube Holder, full speed, 10 min). Pseudo-gel image produced using an Agilent 2100 Bioanalyser show the results of RNA extracted from *Drosophila* (imago) and mosquitos (larva, imago) (A) and from fresh, frozen or NucleoProtect® stabilized mosquito larvae (B). Profile of insect RNA differs significantly from standard profile as the 28S rRNA of most insects contains an endogenous “hidden break” resulting in two similar sized fragments migrating with ~18S rRNA (Krupp, 2005; Winnebeck, Millar and Warman, 2010). Extracted RNA is of high yield and quality based on OD measurements. (C) Lower RIN values in insect RNA measurements result from hidden breaks in insect rRNA assembly.

Ordering information

Product	Specifications	Pack of	REF
NucleoSpin® RNA	Mini spin kit for isolation of RNA of highest integrity	10 preps	740955.10
		50 preps	740955.50
		250 preps	740955.250
NucleoProtect®	RNA stabilization reagent for cells and tissues	50 ml	740400.50
		250 ml	740400.250
		500 ml	740400.500
MN Bead Tubes Type G	2 mL tubes with 5 mm steel beads	50 pieces	740817.50
MN Bead Tube Holder	Rubber-foam adapter for processing MN Bead Tubes with Vortex-Genie 2	1 piece	740469

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