GEroNIMO

Protocol: extraction of total RNA from tissues with NucleoSpin® RNA kit from Macherey-Nagel

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Here we describe the protocol used to purify total RNA from different chicken tissues.

Tissues = liver, magnum, uterus, intestine (jejunum), muscle (Pectoralis), adipose tissue, spleen

To perform the purification of total RNA from the tissue, the User manual of the "NucleoSpin RNA" kit from Macherey Nagel was followed.
(https://www.mn- net.com/media/pdf/b0/51/ee/Instruction-NucleoSpin-RNA.pdf).

1. Protocol description

1.1 Required reagents and instruments

Reagents:

- 96-100% Ethanol (to prepare Wash Buffer RA3)
- 96-100% Ethanol (to adjust RNA binding conditions)
- Reducing agent (β-Mercaptoethanol)

Consumables:

- NucleoSpin® RNA (Macherey-Nagel ref 740955.250)
- 2 mL Tube and 1,5 mL microcentrifuge tubes
- Precellys tissue homogenizing CK14 (reference: P000912-LYSK1-A)
- Pipets and sterile RNAse-free tips
- A cleaning spray against RNAse
- Weighting scales
- Agarose
- TAE (Tris Acetate EDTA) buffer
- Loading buffer for agarose electrophoresis
- Dry ice
- Paper towels
- Waste bag
- Disposable scalpels
- Racks for 2 mL tubes

Equipment:

- Equipement for sample disruption and homogenization (e.g: Homogenizer Retsch MM400)
- Centrifuge for microcentrifuge tubes
- DeNovix spectrophotometer
- Cryoprotection gloves
- Imager

Tissue weight required for the protocol:

- ~ 30 mg for liver
- ~ 45-55 mg for magnum
- ~ 45-55 mg for uterus
- X mg for muscle, intestine, spleen, adipose tissue

1.2 Preparatory step

Before starting the purification of total RNAs, the adequate quantity of tissue was weighted and tissue homogenization was performed. For each frozen sample, X mg of tissue (see above) was excised above a box of dry ice to maintain them frozen. To avoid contamination, a new scalpel between each sample was used. The excised samples are placed into a tube containing beads for homogenization (Precellys Lysing Kit soft tissue homogenizing CK14). To perform tissue homogenization, the Retsch MM400 agitator was used. 350 µL of lysis buffer composed of 346.5 µL of RA1 buffer + 3.5 µL β- mercaptoethanol were added to each tube containing the samples,. We then homogenized the samples by running 2 cycles of 30 sec at 30 Hz for each tube. The lyzed and homogenized sample were transferred to the provided NucleoSpin filter (purple ring) and centrifuged for 1 min at 11000 g.

1.3 Purification of total RNA

1. We transferred the flow through into a new Eppendorf tube and added 350 µL of ethanol 70% to the lysate and mix by pipetting up and down.
2. The NucleoSpin RNA Column was placed in a 2 mL collection tube and the lysate was loaded to the column. We centrifuged for 1 min at 11,000 x g. We discarded the flow-through.
3. 350 µL of MDB Buffer was added to the column and centrifuged for 1 min at 11,000 x g. We discarded the flow-through and reused the collection tube.
4. the DNAse reaction Mixture was prepared in a sterile 1,5 ml microcentrifuge tube: for each isolation 10 µL of reconstituted rDNase was added to 90 µL of reaction buffer for rDNase and mixed by flicking the tube.
5. the 95 µL DNAse reaction mixture was directly applied onto the center of the silica membrane of the column and incubated at room temperature for 15 min.
6. 200 µL of buffer RAW2 was added to the column and centrifuged for 30 sec at 11,000 x g. The flow-through was discarded.
7. 600 µL of buffer RA3 was added to the column and centrifuged for 30 sec at 11,000 x g. The flow-through was discarded and reused the collection tube.
8. 250 µL of buffer RA3 was added to the column and centrifuged for 2 min at 11,000 x g to dry the membrane completely.
9. The Nucleospin RNA column was placed into a new 1.5 mL collection tube. The RNA was eluted in 60 µL of RNase-free water and centrifuged for 1 min at 11,000 x g.

1.4 Storage, quantification and determination of RNA integrity

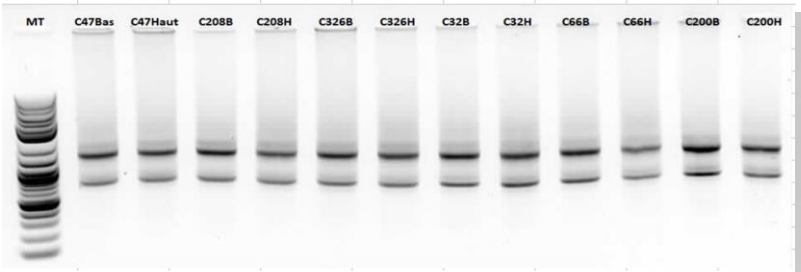
RNAs are stored at - 80°C. Quantification is performed with a spectrophotometer to determine the concentration and the 260/230, 260/280 ratios.
RNAs Integrity were evaluated by migrating 1 *µg total* RNA on 1% agarose gel.

1.5 Storage, quantification and determination of RNA integrity

The total RNA concentration, quantity and DO, obtained on average from X mg of tissue are as follows

- ~30 mg for liver : 760 ng/µL (30 µg) with DO260/230 = 2.1 and DO260/280 = 2.1
  Standard deviation *225 ng/µL* *0.12* *0.01*
- ~45-55 mg for uterus: 520 ng/µL (30 µg) with DO260/230 = 2.2 and DO260/280 = 2
  Standard deviation *72 ng/µL* *0.03* *0.1*
- ~45-55 mg for magnum: 510 ng/µL (30 µg) with DO260/230 = 2.1 and DO260/280 = 2
  Standard deviation *113 ng/µL* *0.16* *0.16*

H=Uterus B=Magnum



Liver