EURx® MOLECULAR BIOLOGY PRODUCTS

Version 1.0
October 2016

fix RNA

Solution for storage and protection from degradation samples prior to RNA isolation.

Cat. no. E0280

DOMINIQUE DUTSCHER SAS

EURx Ltd. 80-297 Gdansk Poland
ul. Przyrodnikow 3, NIP 957-07-05-191
KRS 0000202039, www.eurx.com.pl
orders: email: orders@eurx.com.pl
tel. +48 58 524 06 97, fax +48 58 341 74 23

Table of Contents

DOMINIQUE DUTSCHER SAS

Introductory Notes

NOTE 1 · Kit Specification. ***Fix RNA*** reagent is a ready-to-use solution. ***Fix RNA*** solution rapidly permeats tissues and cells for achieving stabilization and protection of cellular RNA from degradation. The reagent allows to preserve sample material for long term storage and allows efficient isolation of RNA at any time. For efficient protection of RNA, use ***fix RNA*** only on fresh sample material, such as fresh tissue, blood (leukocytes), cell - or bacterial cultures. Do not use frozen samples or samples initially stored without addition of ***fix RNA*** reagent. For protection and stabilizion of whole blood samples we recommend using the **Universal Blood RNA Kit** (E3594) and of **Lyse Blood** buffer included with the kit.

NOTE 2 · Storage of stabilized samples. Samples suspended within ***fix RNA*** solution are stable at room temperature for up to 7 days or at 4–8°C for up to four weeks. Recommended conditions for long-term storage are freezer stocks kept at -20°C or -80°C. For long-term storage at -20°C, prepare the sample as follows: Incubate sample for 8–12 hours within the reagent at 4–8°C, then transfer the sample (with the reagent) to -20°C for storage. Samples stored in ***fix RNA*** solution at -20°C may not freeze. When aiming at -80°C long-term storage, first incubate the sample for 8–12 hours in the reagent at 4–8°C, then remove the sample from the reagent and transfer to -80°C for storage.

NOTE 3 · Maximum Sample Portion. Immediately after harvesting, place the tissue sample in 10 volumes of ***fix RNA*** solution or use approximately 10 µl reagent per 1 mg sample. Pay particular attention to entirely cover the tissue surface with ***fix RNA*** solution. If necessary, larger volumes may be used. To ensure rapid and correct stabilization of RNA, increase the surface-to-volume-ratio by cutting the sample into thin slices, ideally less than 0.5 cm thick. If the slices are too voluminous and the surface-to-volume ratio is consequently rather small, diffusion of ***fix RNA*** solution is either too slow or incomplete, resulting in RNA degradation of slowly or incompletely permeated parts of the tissue sample. For sample material derrived from cell cultures, bacterial cultures or leukocytes, use 10 volumes of ***fix RNA*** solution per one volume of sample (or pellet). Do not use less than 400 µl of ***fix RNA*** solution per sample.

NOTE 4 · Kit Compounds Storage. Once the kit is unpacked, store all components at room temperature. In case of occasional buffer ingredients precipitation, simply warm affected components to about 40°C in a water bath, until the affected solution appears completely clear, without any remaining turbidity. After re-cooling to room temperature, the solution may be used as usual for protecting fresh sample material from RNA degradation.

NOTE 5 · Maintaining Good Working Practice. The solution should be kept tightly closed to avoid evaporation losses, resulting in components concentration changes and precipitation. Avoid introducing any RNases during the procedure or during later handling.

DOMINIQUE DUTSCHER SAS

Isolation of RNA from stabilized samples

Purification of RNA from stabilized samples may be performed using EURx Gene Matrix kits. For extraction and isolation of high-quality, DNA-free RNA, we recommend using **Universal RNA** (E3598), **Universal RNA/miRNA** (E3599) or **Human Blood RNA** (E3596) kits, respectively. To protect, stabilize and isolate RNA from whole blood we recommend **Universal Blood RNA Kit** (E3594). For simultaneous recovery of DNA, RNA and protein from human, animal, plant, bacterial or cell culture samples, we recommend using the **Universal DNA + RNA + Protein Kit** (E3599).

Be sure to separate sample material from **fix RNA** reagent before proceeding with lysis or disruption and homogenization of sample material during the RNA purification procedure. For tissue samples, the sample can be drawn from the solution using sterile tweezers before placing in the appriopriate lysis buffer (buffer **RL**, **LG**, **DRP** or **Lyse ALL** respectively), according to the respective isolation protocol for the kit of choice. In case of particulate, granular consistence of sample material (very small pieces / particles) or for cell cultures, bacteria cultures or leukocytes, proceed as follows: Immediately prior to isolation, add one part of RNase free water to two parts of **fix RNA** reagent. Centrifuge, carefully decant the supernatant and remove any remaining solution by careful pipetting. Add an appriopriate amount of lysis buffer to the pelleted tissue and follow the given protocol for isolation of RNA. For further details, see the respective protocol below.

Animal and plant tissues

1. Excise fresh tissue samples from animals or plants and cut samples into slices less than 0.5 cm thick.

   - *To ensure rapid and correct stabilization of RNA, the sample must be cut into slices less than 0.5 cm thick. If the slices are too voluminous, the reagent will diffuse too slowly or not completely and RNA degradation will occur.*

   - *In case of plant samples with hard tissue or with additional barriers serving for environmental protection (thick skin, waxy coating etc.), attempted treatments with fix RNA solution may result in non-efficient, unsatisfactory protection against RNA degradation.*

2. Place sample material in an appropriate quantity of **fix RNA** reagent. Completely submerge the tissue within the solution in an appropriately sized collection vial. Store in accordance with the instructions given in the introductory notes (see page 3, note 2 Storage of stabilized samples).

   - *Use either 10 volumes of fix RNA solution or approximately 10 µl reagent per 1 mg sample.*

DOMINIQUE DUTSCHER SAS

3. Prior to RNA extraction and isolation, any remains of **fix RNA** solution should be discarded:

   - The sample may be drawn from the solution using sterile tweezers before placing in appriopriate lysis buffer (buffer **RL**, **LG**, **DRP** or **Lyse ALL**) according to the isolation protocol in the selected kit.
   - In case of particulate, granular consistence of sample material (very small pieces / particles), immediately prior to isolation, add one part of RNase free water to two parts of **fix RNA** reagent and centrifuge for 2 min at 10 000 x g. Carefully decant the supernatant and remove any remaining supernatant by gentle pipetting. Add an appriopriate amount of lysis buffer to the pelleted tissue and follow the respective protocol for isolation of RNA.
   - Alternatively, filter the solution containing suspended tissue material through mini-filters, followed by rinsing the retained material with a suitable lysis buffer.

   If tissue has been stored at -80°C, do not allow the sample to thaw prior to transferring the material into an appriopriate lysis buffer.

Cell culture

1. Centrifuge the cell culture sample for 2 min at 5000 x g and carefully decant the supernatant.

   Carefully remove any remaining supernatant by pipetting.

   For RNA extraction / isolation using miniprep kits, do not use more than $5x10^6$ cells per one single preparation. For most purposes, sufficient amounts of RNA are obtained from $0.5–1x10^6$ cells per prep.

2. Add an appropriate quantity of **fix RNA** reagent to the cell pellet. Mix thoroughly. Store in accordance with the instructions given in the introductory notes (see page 3, note 2 Storage of stabilized samples).

   Use 10 volumes of fix RNA solution per one volume of sample (or pellet).

3. Prior to isolation of RNA, any remaining **fix RNA** solution should be discarded. Immediately prior to isolation, add one part of RNase free water to two parts of **fix RNA** reagent and centrifuge for 3 min at 10 000 x g. Carefully decant the supernatant and remove any remaining supernatant by gentle pipetting. According to the isolation protocol for the kit of choice, add an appriopriate lysis buffer (e.g. buffer **RL** or **DRP**) to the pelleted cells (see page 3, Isolation of RNA from stabilized samples).

DOMINIQUE DUTSCHER SAS

Bacteria culture

1. Centrifuge the bacterial culture for 2 min at 10 000 x g and carefully decant the supernatant.

   - *Carefully remove any remaining supernatant by pipetting.*

   - *For RNA isolation using miniprep kits, do not use more than 1x10^9 bacterial cells per single preparation.*

2. Add an appropriate quantity of **fix RNA** reagent to the cell pellet. Mix thoroughly. Store in accordance with the instructions given in the introductory notes (see page 3, note 2 Storage of stabilized samples).

   - *Use 10 volumes of fix RNA solution per one volume of sample (or pellet).*

3. Prior to isolation of RNA, any remaining **fix RNA** solution must be discarded. Centrifuge the sample for 3 min at 10 000 x g. Carefully decant the supernatant and remove any remaining supernatant by gentle pipetting. According to the isolation protocol for the kit of choice, add an appriopriate lysis buffer (buffer **RL** or **DRP**) to the pelleted cells (see page 3, Isolation of RNA from stabilized samples).

   - *Due to the increased density of the solution, centrifugation behaviour of the bacterial cells may deviate from usual conditions. In case of problems during centrifugation of cells, resulting in formation of incomplete or instable cell pellets, simply increase the speed of rotation. Alternatively, add one part of RNase free water to two parts of fix RNA reagent immediately prior to isolation and centrifuge for 3 min at 10 000 x g.*

DOMINIQUE DUTSCHER SAS

Human blood

Leukocytes are components of blood samples, that can be stabilized using ***fix RNA*** solution. Isolation of RNA from fresh or stabilized leukocytes may be performed using the GeneMATRIX Human Blood RNA Purification Kit (E3596). The kit includes all required components for the complete lysis of erythrocytes and for leukocyte separation.

1. Add 4 volumes of buffer **Lyse RBC** to a sample of fresh blood. Mix by inverting the tube.
   - *For example, if the starting blood volume is 300 µl, add 1200 µl of Lyse RBC buffer.*
   - *Lyse RBC buffer is a part of the GeneMATRIX Human Blood RNA Purification Kit (E3596). It is supplied as 5x concentrated stock solution and must be diluted prior to usage.*
   - *Do not use frozen blood (Leukocytes are already lysed due to freezing and thawing).*
2. Keep at 4°C for 10 min for ensuring complete lysis of erythrocytes. Mix twice by inverting the tube.
3. Centrifuge at 400 x g for 10 min at 4°C, and carefully decant the supernatant.
   - *Carefully remove any remaining supernatant by pipetting.*
4. Add two volumes of buffer **Lyse RBC** to the leukocyte pellet. Mix thoroughly by vigorous vortexing.
   - *For example, if the starting blood volume is 300 µl, add 600 µl of Lyse RBC buffer.*
5. Centrifuge at 400 x g for 10 min at 4°C, and carefully decant the supernatant.
   - *Carefully remove any remaining supernatant by pipetting.*
6. Add an appropriate quantity of **fix RNA** reagent to the leukocytes pellet. Mix thoroughly. Store in accordance with the instructions given in the introductory notes (see page 3, note 2 Storage of stabilized samples).
   - *Use 10 volumes of fix RNA solution per one volume of sample (or pellet).*
   - *For RNA isolation using miniprep kits, the maximum blood volume sample amounts to 1.5 ml. Do not attempt to purify from more than 1.5 ml of blood sample per column to avoid any column overloading or column clogging, resulting in incompletely purified RNA.*
7. Prior to isolation of RNA, any remains of **fix RNA** solution must be discarded. Immediately prior to isolation, add one part of RNase free water to two parts of **fix RNA** reagent and centrifuge for 3 min at 10 000 x g. Carefully decant the supernatant and remove any remaining supernatant by gentle pipetting. Pelleted cells should be resuspended in lysis buffer from the GeneMATRIX Human Blood RNA Purification Kit (E3596), according to the instructions given in the kit manual.

DOMINIQUE DUTSCHER SAS

EURx®
MOLECULAR BIOLOGY PRODUCTS

DOMINIQUE DUTSCHER SAS

EURx Ltd. 80-297 Gdansk Poland
ul. Przyrodnikow 3, NIP 957-07-05-191
KRS 0000202039, www.eurx.com.pl
orders: email: orders@eurx.com.pl
tel. +48 58 524 06 97, fax +48 58 341 74 23