# TripleXtractor Reagent

#GB23.0100 (100 preps) | GB23s (trial size)
(FOR RESEARCH ONLY)

**Sample :** Whole blood, buffy coat, cultured animal cells or tissue, cultured plant cells or tissue, cultured bacterial cells, cultured yeast cells.
**Expected Yield :** total RNA (optional: also DNA and/or proteins)
**Operation Time :** within 60 minutes
**Elution Volume :** 10-50µl

**Product:** TripleXtractor is a ready-to-use reagent for the isolation of high-quality total RNA, as well as for the simultaneous extraction of RNA, DNA and protein. TripleXtractor can be used with a wide variety of samples, including blood, buffy coat, cultured cells, animal and plant tissues, bacteria and yeast. During the one-step homogenization/lysis procedure, the strong lysis capability of this monophasic solution of phenol and guanidine isothiocyanate results in the disruption of cells and dissolving of cellular components, while maintaining RNA integrity. The purified total RNA is free of DNA and proteins and can be used for all common downstream applications such as cDNA Library construction, Northern Blotting, RT-PCR, in vitro translation, nuclease protection assays, etc.

During the one-step homogenization/lysis procedure, the strong lysis capability of TripleXtractor results in the disruption of cells and dissolving of cellular components, while maintaining RNA integrity. After addition of chloroform to the extract and subsequent centrifugation, the mixture is separated into an aqueous (upper) phase containing RNA, an interphase, and an organic (lower) phase. The aqueous phase is then transferred to a new tube and the RNA is recovered by Isopropanol precipitation. DNA and/or proteins can be recovered from the sequential precipitation. DNA can be isolated by ethanol precipitation from the interphase, and additional precipitation with isopropanol yields proteins from the organic phase.

**Quantity:** #GB23.0100 consists of 2x 50ml of TripleXtractor. Other reagents that are required or optional and that are not included are chloroform, isopropanol, ethanol, RNase-free water, sodium citrate and TE. #GB23s is a trial sample.

**QC:** TripleXtractor is tested on a lot-to-lot basis by extracting RNA from a 1 ml blood sample. Purified RNA is analyzed by agarose gel electrophoresis.

**Caution:** This product contains phenol and guanidine isothiocyanate. Always work in fume hood and wear protective clothing and goggles. Toxic in contact with skin and if swallowed. Causes burns. After contact with skin or eyes, wash immediately with plenty of water. If you feel unwell, seek medical advice (show label where possible).

In order to prevent RNase contamination, one should use disposable plasticware. Automatic pipettes and non-disposable glassware or plasticware should be sterile/RNase-free and used only for RNA procedures. Glass items can be baked at 150˚C for 4 hours and plastic items can be soaked in 0.5M NaOH for 10 minutes, rinsed thoroughly with RNase-free water and autoclaved. During handling, gloves should be worn at all times.

**Storage:** TripleXtractor should be stored at +2˚C to 25˚C and protect from light. Stable for up to 1 year.

## PROTOCOL FOR RNA ISOLATION

Sample preparation should be done at room temperature. The required volume of TripleXtractor Direct for homogenization and lysis depends on the type of sample.

### 1. HOMOGENIZATION AND LYSIS

#### a. Tissues

Homogenize 50-100mg normal tissue samples in 1 ml of TripleXtractor using a tissue homogenizer or rotor-stator. (Note: Plant tissue 1ml per 15-100mg, Special tissues (spleen, liver, bone: 2ml per 50-100mg). It is recommended to process tissue (from animal or plant, fresh or frozen) with liquid nitrogen and grinding into a fine powder using either a pestle or a mortar, prior to homogenization with TripleXtractor. Incubate for 5 minutes at room temperature and transfer the lysate to a 1.5-ml RNase-free microtube. Proceed with Phase Separation.

#### b. Cells in Suspension

Transfer up to $5x10^6$ cells to a 1.5-ml RNase-free microtube, harvest by centrifugation discard supernatant. Lyse cells in 1 ml of TripleXtractor by pipetting up and down several times. **DO NOT** wash the cells before addition of TripleXtractor as this increases the possibility of mRNA degradation. Incubate for 5 minutes at room temperature. Proceed with Phase Separation.

#### c. Adherent Cells

Remove the culture medium and lyse cells directly in the culture dish by adding 100µl of TripleXtractor per $cm^2$ of culture area and passing the lysate several time through a pipet. Incubate for 5 minutes at room temperature, and transfer the sample to a 1.5-ml RNase-free microtube. Proceed with Phase Separation.
*Note that the amount of TripleXtractor required depends on the area and not on the amount of cells.*

#### d. Blood and Buffy Coat

Transfer up to 300 µl of Blood or Buffy Coat to a 1.5-ml RNase-free microtube and add 3 volumes of TripleXtractor. Mix well by vortexing. Incubate for 5 minutes at room temperature. Proceed with Phase Separation.

#### e. Special Samples (Optional)

Samples with high content of proteins, fat, polysaccharides or extracellular material (e.g., muscle, fat tissue, the tuberous parts of plants) may require an additional centrifugation step. **HOWEVER**, if DNA extraction is required **DO NOT** perform this optional step:

Following homogenization, centrifuge at 14,000g-16,000g for 1 min in order to remove the debris. In case of fat tissue, an excess of fat collects at the top layer and should be removed. In each case, transfer the cleared solution containing RNA to a new RNase-free tube and proceed with the phase separation.

## 2. PHASE SEPARATION

Add 0.2 ml of chloroform per 1 ml of TripleXtractor used. Shake tubes vigorously by hand for 10-15 seconds and incubate at room temperature for 2 to 3 minutes. Centrifuge at 14,000g-16,000g at 2-8˚C for 10-15 minutes. This results in the separation into a lower red phenol-chloroform phase, an interphase (white), and a colourless upper aqueous phase. RNA remains exclusively in the aqueous phase, which corresponds to approximately half of the total volume of TripleXtractor used.

## 3. ISOPROPANOL PRECIPITATION

Transfer the aqueous solution to a new RNase free tube. Avoid carry-over from the interphase or organic phase. If desired, save the interphase and organic phase for subsequent DNA and/or protein isolation.

Precipitate the RNA by adding 1 volume of isopropanol per 1 volume of TripleXtractor used. Mix well by inverting the tube several times. Incubate the samples at room temperature for 10 minutes. Then centrifuge at 14,000g-16,000g at 2-8ºC for 10 minutes. The RNA precipitate forms a gel-like thigh pellet and may be difficult to see

## 4. WASHING

Using a pipet, carefully remove and discard the supernatant avoiding to touch the pellet. Wash the pellet by adding 1 ml of 70% ethanol (prepared with RNase-free water). Very gently invert the tube a few times**. DO NOT VORTEX**. Centrifuge at 14,000g-16,000g at 2-8ºC for 5 minutes. Using a pipet, carefully remove and discard the ethanol and repeat the washing step.

## 5. RNA RESUSPENSION

After the final washing step, discard the ethanol and air-dry for 5 to 10 minutes in order to remove trace amounts of ethanol. It is important not to dry the pellet completely, as this will decrease the RNA solubility considerably. Dissolve the RNA pellet in a desired volume of RNase-free water (e.g., 20-50µl) by pipetting the solution a few times up and down and by incubating at 55-60˚C for 10 minutes. The isolated RNA can be used immediately or stored at -70˚C for later use.

## 6. ANALYSIS

Using a 10 µl microcuvette, read OD at 260nm and at 280nm of a 40-times dilution (e.g., dilute 1 µl of isolated RNA in 40 µl of RNase-free water) to determine sample concentration and purity. Note that a concentration of 40 µg/ml of RNA corresponds to an OD at 260nm of 1.0 and that the A260/A280 ratio should be approximately 2.0, whereas the A260/A230 ratio should be higher and be in the range of 2.0-2.2.

## PROTOCOL FOR DNA ISOLATION

Following phase separation of the RNA isolation protocol (step 2 on page 3), DNA can be recovered from the interphase (white) and the organic phase (red) after removal of the aqueous phase (colourless) by ethanol precipitation.

### 1. DNA PRECIPITATION

Using a pipet, carefully remove any residual aqueous phase layer (upper colourless layer), This is a critical step for DNA quality! Add 300 µl of absolute ethanol per 1 ml of TripleXtractor used during sample homogenization and lysis to the combined interphase and organic phase. Mix well by inverting the tube several times. Incubate at room temperature for 5 minutes and centrifuge the sample at 2,000g at 2-8°C for 5 minutes. Using a pipet, remove the supernatant. Either discard the supernatant or save it at 2-8°C for protein purification.

### 2. WASHING

Add 1 ml of 0.1 M sodium citrate in 10% ethanol, pH 8.5 per 1 ml of TripleXtractor used during the initial sample lysis step. Incubate the sample at room temperature for 30 minutes. During incubation, regularly invert the tube gently. Centrifuge the sample 2,000g at 2-8°C for 5 minutes. Using a pipet, remove and discard the supernatant. Repeat the washing step. Add 1.5 ml of 70% ethanol to the sample per 1 ml of TripleXtractor used. Incubate for 15 minutes at room temperature. During incubation, regularly invert the tube gently. Centrifuge the sample 2,000g at 2-8°C for 5 minutes. Using a pipet, remove and discard the supernatant.

### 3. DNA RESUSPENSION

After the final washing step, discard the ethanol and air-dry for 5 to 10 minutes in order to remove trace amounts of ethanol. It is important not to dry the pellet completely. Dissolve the DNA pellet in a desired volume of TE, pH 8.5 (e.g., 300µl) by pipetting the solution a few times up and down and by incubating at 55-60˚C for 10 minutes. Centrifuge at 14,000g-16,000g for 10 minutes to remove insoluble particles. Transfer the supernatant containing the DNA to a new 1.5-ml microtube. The isolated DNA can be used immediately or stored at -20˚C for later use. Note: if desired, instead of using TE, one can use other weak base solutions such as 8mM NaOH, pH 8.5 to resuspend the DNA pellet.

## PROTOCOL FOR PROTEIN ISOLATION

### 1. PROTEIN PRECIPITATION

Add 1.5 ml of isopropanol to the phenol-ethanol supernatant saved from the DNA precipitation procedure (see above). Mix well by inverting the tube several times. Incubate at room temperature for 10 minutes. In order to precipitate the protein fraction, centrifuge the sample at 14,000g-16,000g for 10 minutes at +4°C. Then carefully remove and discard the supernatant.

### 2. WASHING

Add for each 1 ml of tripleXtractor initially used a total of 2 ml of 0.3 M guanidine hydrochloride in 95% ethanol to the protein pellet, and incubate at room temperature for 20 minutes. The protein pellet could be stored in this wash/storage solution for up to one year at -20°C. Centrifuge the sample at 7,000g-8,000g for 5 minutes at +4°C, then remove and discard the supernatant. Repeat this washing procedure twice.

### 3. PROTEIN RESUSPENSION

Add 200µl if a 1% SDS solution in water to the protein pellet and resuspend by pipetting up and down. Incubate at 50°C for 10 minutes to completely dissolve the protein pellet. Centrifuge at 10,000g for 10 minutes at +4°C to remove debris. Transfer the cleared supernatant to a new 1.5-ml microtube and the protein is ready for downstream applications or storage (at -20°C).