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1. Description

Components	10×0.2 mL SuperAmp™ Tubes 1 SuperAmp Shipment Tube Container 70 µL SuperAmp Shipping Buffer
Size	Reagents for the cell lysis and shipment of 10 samples.
Storage	Store SuperAmp Shipment Buffer protected from light at -20 °C. The buffer is stable until the date indicated on the label. Do not thaw SuperAmp Shipment Buffer more than twice. To avoid multiple freezing and thawing, store the SuperAmp Shipment Buffer in appropriate aliquots. Store the aliquots at -20 °C. Store the lysed samples at -80 °C.

1.1 Principle of the SuperAmp™ Technology

The SuperAmp™ Technology overcomes the problem of limiting amounts of starting material for microarray hybridization. The SuperAmp Procedure amplifies RNA from 1 to 10,000 cells or from comparable amounts of tissue. The SuperAmp Shipment Buffer Kit is intended to store and ship cell aliquots prior to amplifying the RNA by SuperAmp Protocol. Cell aliquots of 1 to 10,000 cells are lysed using the SuperAmp Shipment Buffer and can then be stored at -80°C. The main application of the SuperAmp Shipping Buffer Kit is the possibility to send the samples on dry ice in case the amplification will be performed in another laboratory.

1.2 Background information

The analysis of gene expression is widely used for many purposes including the characterization of cells, the study of diseases, or the identification of drug targets. However, there are experimental settings where it is not possible to obtain more than a few cells, for instance when surgical biopsies or rare cell subsets are analyzed. Under these circumstances, protocols for the efficient isolation and millionfold amplification of picogram amounts of RNA are needed. Based on the well-established MACS® Technology the SuperAmp

Procedure allows highly sensitive mRNA isolation, cDNA synthesis and millionfold amplification of mRNA-derived cDNA by global polymerase chain reaction.

The unique SuperAmp Protocol combines several features:

- Extremely small (50 nm) superparamagnetic MACS MicroBeads instantly bind and label mRNA molecules from small samples
- MACS Column Technology simplifies the required washing steps resulting in highly pure mRNA
- In-column cDNA synthesis and purification reduces loss of individual transcripts
- Small volumes facilitate optimal reaction kinetics
- Generated cDNA fragments have a uniform size to enable homogenous PCR amplification.
- Global PCR is driven by a single primer to ensure uniform amplification due to consistent annealing conditions.

The SuperAmp Shipment Buffer provided with the SuperAmp Shipment Buffer Kit allows a very efficient cell lysis. The buffer is optimized for bead-based direct mRNA isolation and reverse transcription. All reagents and procedures have been established and optimized with the tubes included in the kit. SuperAmp can be used to analyze tissues and cells which have been isolated with diverse methods, for example, MACS® Technology, laser capture microdissection, or FACS.

1.3 Applications

- Lysis, storage, and shipment of tissues and cells for subsequent mRNA amplification via SuperAmp Technology.

1.4 Reagent and instrument requirements

- ▲ The benchtop must be free of RNase and contaminating DNA or RNA.
- RNase-free pipette tips.
- Water bath or heat block at 45 °C.
- Microcentrifuge suitable for 0.2 mL tubes (or use 2 mL tubes as centrifuge adaptors).

2. Protocols for lysis and storage of samples

2.1 Before starting

- ▲ The SuperAmp™ Procedure allows the amplification of mRNA from single cells. Therefore, the procedure is extremely sensitive to RNA or DNA contaminations. Make sure that all instruments are free from RNases and contaminating RNA or DNA, and that they are not contaminated during the isolation or dissection of tissue or cells. For example, when cytoplasm from single cells is isolated, make sure that the pipette tip does not touch other dead or living cells.
- ▲ The SuperAmp Procedure requires supply of samples in the provided SuperAmp Tubes. The cells to be analyzed are lysed in 6.4 µL

Lysis Buffer. The volume of the cells before being lysed should be adjusted to 1 μL with no more than 10,000 cells/ μL . Cell suspensions containing more than 10,000 cells/ μL may not be lysed efficiently leading to a reduced integrity of RNA. If ever possible, samples should be lysed directly in these SuperAmp Tubes. Transferring sample from tube to tube will lead to loss of material

▲ Mark the SuperAmp Tubes on the top and on the side wall e.g. by using numbers or abbreviations. Please make sure that the labels are legible.

▲ If sending multiple samples, please make sure that the amount of cells is approximately the same in all samples.

2.2 Sample preparation

▲ Heat water bath, PCR block, heating block, or incubator to 45 °C.

▲ Thaw the SuperAmp Shipment Buffer at room temperature and spin down.

▲ The volume of the cells before lysis must not exceed 1 μL . The cell suspensions should not contain more than 10,000 cells/ μL . Cell suspensions containing more than 10,000 cells/ μL may lead to inefficient lysis.

2.2.1 Preparation of liquid samples with less than 10,000 cells in a maximum volume of 1 μL

Directly start with step 1 in chapter 2.3, Cell lysis.

2.2.2 Preparation of liquid samples with less than 10,000 cells in a volume of 1 μL to 200 μL

▲ Pre-cool centrifuge to 4 °C.

1. Collect the cells (e.g. from FACS™ or MACS Cell Separation), ideally in ice-cold PBS, in the provided 0.2 mL tube.
2. Pellet the cells by centrifuging at 300 \times g for 10 minutes at 4 °C. Carefully aspirate the supernatant leaving the cells in approximately 1 μL .
3. Continue with step 1 in chapter 2.3, Cell lysis.

2.2.3 Preparation of liquid samples with a volume larger than 200 μL and/or a cell concentration higher than 10,000 cells/ μL

▲ Pre-cool centrifuge to 4 °C.

1. Collect the cells, ideally in ice-cold PBS, in an appropriate tube for centrifugation.
2. Measure cell concentration.
3. Pellet the cells by centrifuging at 300 \times g for 10 minutes at 4 °C. Carefully aspirate the supernatant.
4. Resuspend the cells in an appropriate volume of PBS to a concentration of max. 10,000 cells/ μL .
5. Continue with step 1 in chapter 2.3, Cell lysis.

2.2.4 Preparation and lysis of dry samples from microdissected tissue

1. Collect the cells in a 0.2 mL tube as suggested by the supplier of the laser microdissection device.
▲ **Note:** Do not microdissect more than 10,000 cells per tube.
2. Add 6.4 μL freshly prepared Incubation Buffer (refer to chapter 2.1.2) to the microdissected cells. Resuspend the cells by pipetting up and down 10–20 times.
3. Incubate at 45 °C for 10 minutes in a thermocycler. If cells have been trapped in the tube cap, make sure that incubation temperature in the cap is 45 °C by heating the lid of the thermocycler to 45 °C. Spin down liquid at 2500 \times g.
4. Incubate tube in a thermocycler for 1 minute at 75 °C and briefly spin down liquid at 1,000 \times g.
5. Freeze the lysed cell aliquots in SuperAmp™ Shipping Buffer at –80 °C. To ship the samples, send the cell lysates on dry ice.

2.3 Cell lysis

▲ Assure that cell suspension is always on ice **before** adding the SuperAmp Shipment Buffer to prevent RNA degradation.

1. Add 5.4 μL of the SuperAmp Shipment Buffer Buffer to the tube containing 1 μL cell suspension. Vortex cell suspension thoroughly and briefly spin down liquid at 1,000 \times g.
2. Place tube in water bath, PCR block, heating block, or incubator heated to 45 °C and incubate 10 minutes at 45 °C followed by 1 minute at 75 °C.
3. Freeze the lysed cell aliquots at –80 °C.
4. Insert the frozen tubes in the SuperAmp Shipment Tube Container to prevent breakage of the tubes during shipment.
5. Send SuperAmp Shipment Tube Container including the samples on dry ice.

Warranty

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