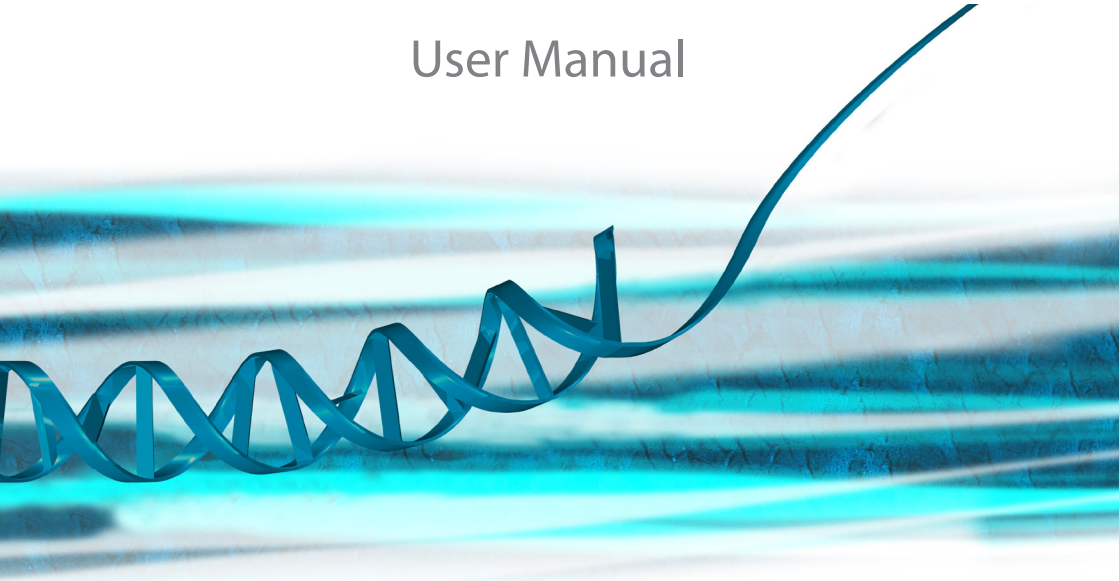
User Manual



CelluLyser™ Micro
Lysis and cDNA Synthesis Kit

Version 1.1— Dec 2013

From cells to cDNA in one tube

tataabiocenter

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Introduction

The CelluLyser™ Micro lysis buffer has been de novo designed to provide improved mRNA retrieval even for very low expressed genes, enhanced stabilization of mRNA, minimal degree of dilution and high compatibility with downstream reagents. These properties make direct lysis with CelluLyser™ Micro and analysis of single cells more accurate, flexible and feasible. The CelluLyser™ Micro lysis reagent has been developed for fast and simple cDNA preparation for single cell applications including very small samples (FACS sorted single cells, cell cultures, dissociated tissue). By generating cDNA directly from cell lysate without any washing steps, higher yields are achieved, no bias in transcriptome is introduced and considerable amount of time is saved compared to standard column based purification (Svec *et al.,* 2013). To avoid losses of material the entire procedure can be performed in a single tube (or 96/384-well plates) without any sample transfer, just adding reagents. The whole process can be easily automated for high-throughput applications.

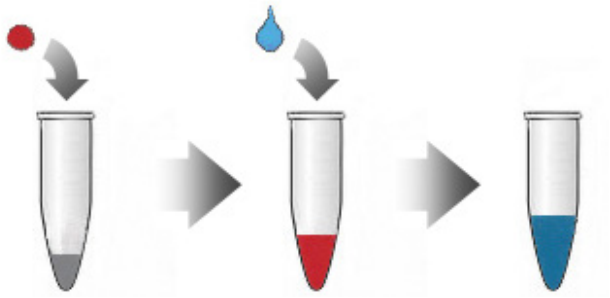


Figure 1: Cells are lysed in the CelluLyser™ Micro buffer and reverse transcribed by adding TATAA GrandScript cDNA synthesis kit directly to the cell lysate. After incubation the cDNA sample is ready for downstream qPCR.

CelluLyser™ Micro is:

- **Lysis buffer optimized for direct cell lysis from a single cell up to 500 cells in only 5 µl volume**
- **Optimized to preserve and stabilize released RNA**
- **Compatible with downstream reagents for reverse transcription**
- **Compatible with TATAA Universal RNA and DNA spikes for quality control of the workflow**

Contents

- CelluLyser™ Micro buffer: 200 rxn à 5 µl lysis
  1.0 ml of 1X concentration
- GrandScript cDNA synthesis kit: 200 rxn à 10 µl RT
  400 µl GrandScript RT Reaction Mix (5x)
  100 µl GrandScript RT enzyme
  Nuclease free water

Storage

CelluLyser™ Micro lysis buffer may be stored in 4°C for up to two months. For long term storage keep at -20°C. The TATAA GrandScript cDNA synthesis kit components should be stored at -20°C. Avoid repeated freeze-thaw cycles. Lysed RNA stored in CelluLyser™ Micro buffer shows very low decay in room temperature as well as after freeze thaw cycles. However, it is recommended to store sample material in -80°C if cDNA synthesis is not performed within 6 hours from the lysis.

Additional materials and equipment required

• General laboratory equipment including vortex and microcentrifuge.
• Pipettes, nuclease-free pipette tips and microcentrifuge tubes (availiable from www.tataa.com/products).

Lysis - additional information

Number of cells: Tested on several cell types, the maximum number of effectively lysed cells depends on the volume of CelluLyser™ Micro. Normally 5 µl is the minimum volume for most applications using FACS sorting in 96 well plates. Maximum number of cells which are effectively lysed is 500 cells, if more than 500 cells are used the linear response may become compromised.

gDNA background: A separate DNase treatment is optional. The level of genomic contamination is minimal in single cell profiling, however, to follow MIQE guidelines (Bustin *et al*., 2009), the standard RT(-) control (ΔCq≥5) or novel, cost saving ValidPrime approach (Laurell *et al*. 2012) can be used.

Reverse Transcription: The concentration of all reagents in the CelluLyser™ Micro buffer has been adjusted to achieve a non-inhibitory level in reverse transcription if the lysate represents maximum 50% of RT volume. If there is a risk of transferring inhibitors together with the cells, we recommend to validate purity of samples using Universal RNA spike I or II (Svec *et al*. ,2013), available at www.tataa.com.

Protocol - Lysis

The protocol is optimized for use with 1-500 cells in 5µl. Using >500 cells may result in incomplete lysis and/or inhibition of cDNA synthesis. The cell lysis can be performed in either 0.2 ml PCR tubes, 96- or 384-well cell culture plates.

Cell sorting

1. Add 5µl Cellulyser™ Micro lysis buffer into a tube or well.

2. Sort the cell(s) into the CelluLyser Micro™ buffer. Collect the cell(s) with a minimal amount of residual liquid and add it directly to the lysis solution. During the collection the cells should be kept in a medium or buffer that is not inhibitory for downstream reactions, e.g. phosphate buffered saline (PBS) buffer. Multiple cells may be added to the tube or well as long as the amount of residual liquid is low enough to avoid any significant dilution of the Cellulyser™ Micro (max. additional 5%), which may cause incomplete lysis of the cells.

Cell suspension

1. Pellet the cells and wash the pellet with 4°C PBS buffer.

2. Carefully remove the PBS buffer without disturbing the cells. It is critical to remove as much PBS buffer as possible as remaining buffer will dilute the lysis solution and may cause incomplete lysis (max. leftover is 5%).

3. Dissolve the cell pellet in 5µl of CelluLyser™ Micro lysis buffer.

Adherent cells grown in 96 or 384 well cell culture plates

1. Aspirate the culture media from each well. Wash the cells with 4°C PBS buffer.

2. Carefully remove the PBS buffer without disturbing the cells. It is critical to remove as much PBS buffer as possible as remaining buffer will dilute the lysis solution and may cause incomplete lysis (max. leftover is 5%).

3. Add 5µl Cellulyser™ Micro lysis buffer to each well (make sure to cover all cells if using flat bottom wells).

Cell types

The CelluLyser™ Micro buffer has been developed to be a mild lysis solution. It has been verified on many types of in vitro and in vivo viable cells including astrocytes, microglia, fibroblasts, lymphocytes (B-cells, T-cells), leukemia cell lines (K562, HEK293), monocytes, breast cancer cells, sarcoma cell lines and embryonic stem cells. However, if more robust cells are used or if complete lysis is not achieved the alternative stronger lysis solution CelluLyser™ together with Transcriptor RT™ could be evaluated (www.tataa.com).

Protocol - Reverse Transcription

Reverse Transcription

Before use, thaw all frozen components. Mix thoroughly and briefly centrifuge to collect contents. Place all components including the TATAA GrandScript RT enzyme on ice.

1. Add the following to each 5 µl CelluLyser™ Micro lysis solution in a 0.2 ml thin walled PCR tubes, or in a 96 or 384-well PCR plate.

Nuclease free water	**2.5 µl**
TATAA GrandScript RT Reaction Mix (5x)	**2.0 µl**
TATAA GrandScript RT Enzyme	**0.5 µl**
Final volume	**10.0 µl**

2. Vortex gently and centrifuge to collect content

3. Incubate the tube(s) under the following conditions

22°C, 5 min
42°C, 30 min
85°C, 5 min
(4°C, hold)

4. After cDNA synthesis, use 10-20% of the first strand reaction in subsequent qPCR (2-4 µl cDNA for a 20 µl qPCR). At this point the synthesized cDNA may be stored at 2 to 8°C for several hours or at -15°C to -25°C for longer periods.

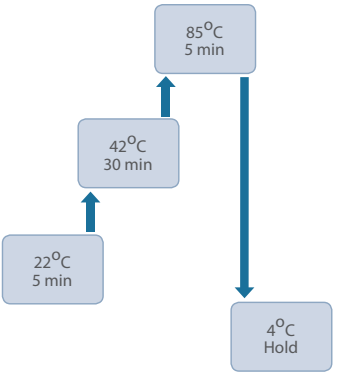


Figure 2: Heating protocol for cDNA synthesis using TATAA GrandScript cDNA synthesis kit.

Troubleshooting

No amplification

Not being able to amplify any genes in your material could be due to incomplete cell lysis. After careful washing of cells, make sure not to bring in more than 5% remaining PBS buffer into the lysis step, as more PBS buffer will dilute the lysis solution and may cause incomplete lysis.

Another explanation for no amplification could be no presence of cell material in the sample. If collecting cell material with FACS sorting make sure correct settings are used in order for the cell containing droplets to successfully reach the lysis solution. The droplet impact zone can be tested by estimating the flight track of the droplets when sorting droplets onto a sealing film on the plate. It can also be tested by using a Universal RNA spike (www.tataa.com). Sort the RNA spiked droplets into the CelluLyser™ Micro lysis byffer and analyse the presence of spike with RT-qPCR.

Verify that the qPCR instrument is set to collect data in the appropriate channel for the dye or probe chemistry used. A positive qPCR control such as a Spike control or the TATAA Interplate calibrator could be used.

High Cq values or no amplification due to inhibition

Inhibitors from sample matrix might interfere with RT efficiency and/or qPCR efficiency, resulting in high Cq values or no amplification. Test the presence of inhibitors in the RT by adding an RNA Spike and test the qPCR by adding a DNA Spike to the sample. Compare the result with a spike added to pure nuclease-free water.

Also make sure not to add more than 10-20% cDNA into the qPCR volume, as adding more might inhibit the qPCR reaction.

Non-specific amplification

Non-specific amplification such as primer-dimer formation will interfere with correct data collection when using dye-based chemistry or lower the qPCR efficiency when using probe based chemistry. Perform a melt curve analysis for dye based chemistry or run your PCR product on a gel to ensure only your target is being amplified in the qPCR.

High variation between biological replicates

Make sure an amount of less or equal to 500 cells is used. Using more than 500 cells per lysis reaction may result in incomplecte lysis and/or inhibiton of cDNA synthesis.

If samples have not been stored properly there is a risk of degradation of the RNA or cDNA which will result in unreliable data. The lysate may be kept at 4°C for a few hours or at -80°C for long term storage. The synthesized cDNA may be stored at 4°C for several hours or at -15°C to -25°C for longer periods.

The CelluLyser™ Micro lysis buffer should be equilibrated to room temperature before lysis of cells to ensure homogenous treatment of cells.

High variation between technical replicates

Validate the pipettes used and make sure compatible pipette tips are used.

It is highly recommended to validate both commercial and in-house developed assays for efficient and robust performance, especially when analyzing single cells. Very low expressed targets giving rise to high Cq-values might result in a large spread of replicates. Consider preamplifying your material prior to qPCR analysis.

Positive NTC

A positive signal from the no template control (NTC) indicates contamination of reagents or equipment by PCR product or sample. Solve this by decontaminating the equipment and change for new contaminats-free reagents.

Positive NoRT/RT(-) control

Signal from the NoRT/RT(-) control indicates either presence of genomic DNA or contaminating DNA in the sample. Try to use assays designed to span exon-exon boundaries or perform an optional DNase treatment using a heat-labile DNase. Also consider to decontaminate the equipment and change for new contaminats-free reagents.

Support

Please contact us at info@tataa.com for further support.

Contact and ordering information

To reorder or for more information about the product and other products available from TATAA Biocenter, please contact us on order@tataa.com or visit our website www.tataa.com.

CelluLyser™ Micro Lysis and cDNA Synthesis Kit	200 rxn	Order#	H103
CelluLyser™ Micro Lysis buffer	200 rxn	Order#	H104

References

Bustin SA, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M, Mueller R, Nolan T, Pfaffl MW, Shipley GL, Vandesompele J, Wittwer CT; The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clin Chem*. 2009 Apr;55(4):611-22. Epub 2009 Feb 26.

Laurell H, Iacovoni JS, Abot A, Svec D, Maoret J-J, Arnal J-F, Kubista M; Correction of RT–qPCR data for genomic DNA-derived signals with ValidPrime. *Nucleic Acids Res*. 2012 April; 40(7): e51.

Svec D, Andersson D, Pekny M, Sjöback R, Kubista M, Ståhlberg A; Direct cell lysis for single-cell gene expression profiling. *Frontiers of oncology* 2013

Safety information

When working with chemicals, always wear a protective lab coat, disposable gloves, and protective eyewear. See the appropriate material safety sheets (MSDS) for more information.

License information

Other products from TATAA

CelluLyser™ lysis and cDNA synthesis kit

This lysis and cDNA synthesis kit is optimized for 500-10000 cells or for cells that are more diffucult to lyse. This slightly stronger lysis buffer is developed to be compatible with the robust Transcriptor RT™ cDNA synthesis kit.

TATAA PreAmp GrandMaster® mix

The TATAA PreAmp GrandMaster® Mix is a 2x concentrated ready to use mix for PCR based preamplification of small samples, when looking at low expressed genes or when highly concentrated RNA is needed. The mix provides all the necessary components for preamplification (except template and primers).

TATAA SYBR® GrandMaster® mix

TATAA SYBR® GrandMaster® Mix is a 2x concentrated ready to use fastmix. With its unique combination of optimized components it delivers maximum PCR efficiency, sensitivity, specificity and robust fluorescent signal using fast, or conventional, cycling protocols with SYBR® Green qPCR.

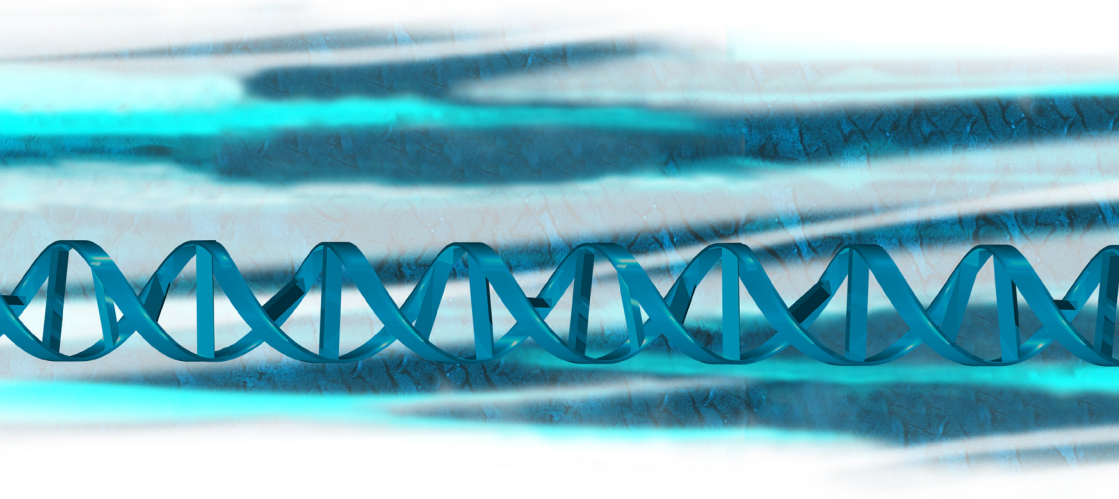
TATAA Probe GrandMaster® mix

The TATAA Probe GrandMaster® Mix is a 2x concentrated ready to use fastmix, with a clear blue color that facilitates pipetting in clear and white plates. The TATAA Probe GrandMaster® Mix provides a robust and versatile solution for ultimate sensitivity and efficiency. It is compatible with a variety of fluorogenic probe chemistries including TaqMan® hydrolysis probes and contains an ultra pure, processive thermostable DNA polymerase that is free of detectable E. coli DNA.

Universal RNA/DNA spike - tests for inhibition and yield

The TATAA Universal Spike is an easy to use and very effective tool for quality control throughout the entire RT-qPCR experimental workflow. Add the Spike to the experimental sample and to a control based on water. Processing both samples exactly the same way – any inhibition in the experimental sample will impair the RT-qPCR resulting in higher Cq than of the control sample. TATAA Universal Spikes have an artificial sequence that is not present in any known living organism. The Spike assay is exceedingly robust and is optimized for high sensitivity for inhibition. The Cq of the Spike assay also reflects losses during extraction, handling, transport, and storage of samples, including freeze-thaw events during RT-qPCR.

Express your genius



TATAA Biocenter, with offices in Gothenburg, San Francisco and Prague is the leading provider of real-time PCR services and the prime organizer of real-time PCR workshops globally. TATAA Biocenter conducts commissioned research and training within the field of molecular diagnostics and gene expression analysis, along with developing real-time PCR expression panels. TATAA Biocenter has great experience and expertise in high resolution gene expression profiling, pathogen detection, and small sample/single cell analysis.

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