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Protein Sample Preparation in Eppendorf Tubes® 5.0 mL

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Abstract

Analysis of proteins from cultured cells often requires sample processing at a larger volume scale. Examples include isolation of cell fractions from larger homogenates, determination of enzyme activities as well as harvesting of proteins from sparsely growing cells.
The new Eppendorf Tubes 5.0 mL are the ideal vessel format for these applications. Their use allows larger processing volumes, high centrifugation forces and improved handling.

Introduction

Over the past 50 years reaction tubes in the microliter range have become the mainstay in the laboratory with volume formats of 0.5 mL, 1.5 mL and 2.0 mL used most frequently. The recent introduction of the new format of 5.0 mL tubes has opened up new applicative options.
Especially in the field of analysis of proteins from cultured cells, many applications require protein processing at a larger volume scale. Examples include isolation of cell fractions from larger homogenates, determination of enzyme activities as well as harvesting of proteins from sparsely growing cells or comparative immunoblot analysis of total protein isolates.
The new Eppendorf Tubes 5.0 mL are ideally suited for such applications; their use allows large processing volumes and high centrifugation forces as well as easy and convenient one-handed operation. In this Application Note we have investigated the performance of the Eppendorf 5.0 mL tube format by addressing the following questions:
1) Are the required steps easily adaptable to the new format and do temperature differences during processing of larger volumes present a problem?
2) Does the use of different tube formats lead to quantitative differences (e.g. caused by sample loss)?
3) Does the use of the larger tube format result in compromised protein quality or activity?
To investigate these questions, a comparative analysis for isolation and preparation of protein samples was performed by using different tube volume formats.

Materials and Methods

Comparative cell lysate preparation for immunoblot analysis

Human colon carcinoma cells (HT29) were seeded into five 15 cm cell culture dishes and grown to a confluence of approximately 70 %. Following aspiration of the culture medium (DMEM, 10 % FBS, P/S) the cells were washed once in PBS and subsequently scraped off using 600 µL lysis buffer per plate (Cell Signaling Technology®, Inc.). The combined cell suspensions were pooled in one 50 mL tube. The volume was then adjusted to a total of 20 mL using lysis buffer, and the cells were thoroughly resuspended and distributed among several different types of reaction vessels in order to enable subsequent comparative preparations: Eppendorf Safe-Lock Tubes 1.5 mL and 2.0 mL, as well as Eppendorf Tubes 5.0 mL. Following filling of the reaction tubes, samples were immediately snap-frozen in liquid nitrogen* and stored over night at -80 °C. The samples were thawed on ice. Destruction of cell membranes was achieved by sonication rod (3 cycles, on ice). The membrane debris was separated by centrifugation (3,200 x *g*, 4 °C, 10 min) and the supernatant was transferred to new reaction tubes. Quality of the cell lysates was determined by total protein quantification using the BCA method. In accordance with the manufacturer's instructions, protein samples were incubated with BCA (50 parts reagent A + 1 part reagent B) for 30 minutes at 37 °C and absorbance was measured at 562 nm in a plate reader.

Determination of aconitase activity

Murine embryo fibroblasts from two 15 cm cell culture dishes, each approximately 50 % confluent, were washed once with Tris buffer (50 mM) and subsequently scraped off using 600 µL Tris buffer per plate. The volumes obtained (approximately 2.8 mL) were transferred to one 5.0 mL or two 1.5 mL Eppendorf tubes, respectively, resuspended and snap-frozen in liquid nitrogen*. Following storage at -80 °C the samples were thawed on ice and sonicated. Membrane debris was removed by centrifugation. The supernatant with cleared lysate was then transferred to new reaction tubes and total protein concentrations were determined using the Bradford method (approximately 1.1 µg/µL). Eight aliquots containing 130 µg of protein each were diluted to a volume of 150 µL using Tris buffer. 150 µL of assay buffer (50 mM Tris, 60 mM sodium citrate, 1 mM $MnCl_2$, 0.4 mM NADP+) were pipetted into eight wells of a 96 well micro plate, followed by addition of 150 µL protein solution. Four of the eight reactions received reactivation substances (dithiothreitol, Na_2S, Mohr's salt). The formation of NADPH was measured over a period of 60 minutes in a plate reader (37 °C, 340 nm) and the relative aconitase activity was calculated accordingly.

Results and Discussion

Hardly any other application places a higher demand on consumables than isolation and processing of protein samples for immunoblot analysis. The tubes must withstand a broad temperature range and high centrifugal forces, while at the same time protecting the samples and remaining easy to open and close. In this application we compared the use of different Eppendorf tube formats (1.5 mL, 2.0 mL and 5.0 mL) for immunoblot analysis. In Table 1 the main sample processing steps are compared.

	Eppendorf Safe-Lock Tubes 1.5 mL	Eppendorf Safe-Lock Tubes 2.0 mL	Eppendorf Tubes 5.0 mL
Fill volume with cell suspenson (approximately 2/3 of the maximum volume)	1.0 mL	1.3 mL	3.3 mL
Time until thawed on ice	60 min	70 min	90 min
Volume transferred following centrifugation	0.9 mL	1.2 mL	3.0 mL
Addition of LAEMMLI buffer	0.45 mL	0.6 mL	1.5 mL

Table 1: Comparison of sample processing steps for immunoblot analysis using different Eppendorf tube formats: 1.5 mL, 2.0 mL and 5.0 mL.

Details of the comparative sample processing comprised the following: first, a homogeneous cell suspension was prepared and split into different vessels, filling 2/3 of the maximum volume. All samples were snap-frozen in liquid nitrogen* and stored over night at -80 °C. As expected, thawing on ice using larger reaction tubes required more time until further processing was possible. The larger conical shape of the 5.0 mL tubes proved optimal for homogenization with a rod sonicator and general handling was very convenient.
During this step, the Eppendorf Tubes 5.0 mL display clear advantages over the smaller tube-formats; the danger of sample material spillage from the tube is limited, and sample loss by adhesion to the sonication rod is reduced as compared to smaller tube-formats.
Various rotors and accessories available for the Eppendorf Tubes 5.0 mL allow easy fit with most centrifuges and application of high centrifugal forces (up to 25,000 x *g*).
In our experiment pelleting the cellular debris was performed at 3,200 x *g* and the cleared supernatant was transferred to fresh reaction tubes. Processing in larger tubes also offered the clear advantages of improved handling and better sample retrieval; removing the supernatant was prone to less contamination and it was also quicker.
In order to control for quality of the cell lysate, a portion was used for total protein quantification with the BCA method. As shown in Figure 1, no significant differences in protein concentrations between different vessel formats could be detected. These data show that processing larger volumes in the 5.0 mL format does not lead to a quantitative sample loss.

The remaining portions of the samples were directly used for electrophoresis and subsequent immunoblot analysis (data not shown).
Furthermore, in order to investigate whether using the 5.0 mL format might compromise protein quality a comparison of enzymatic activity (aconitase) was performed in cell lysates processed in 1.5 mL and 5.0 mL tubes.
No differences could be detected in either reactivated or non-reactivated enzyme preparations when compared between 1.5 mL and 5.0 mL tubes (data not shown). This demonstrates that the processing of samples in Eppendorf Tubes 5.0 mL influences neither the quality nor the activity of proteins in the tested cell lysates.

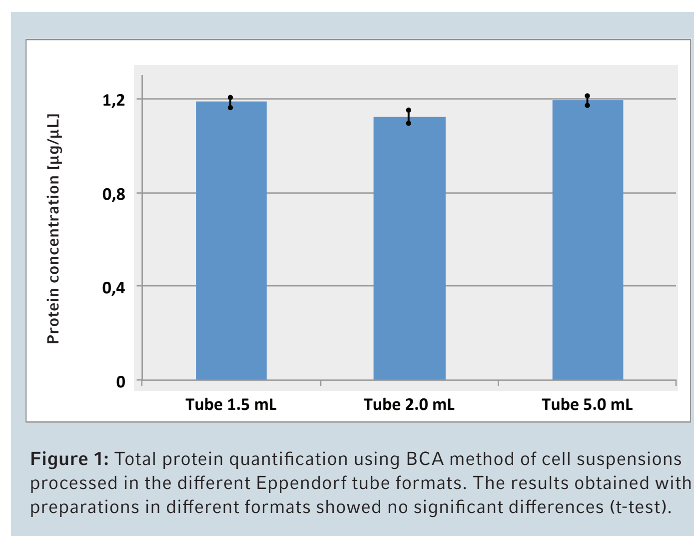

Figure 1: Total protein quantification using BCA method of cell suspensions processed in the different Eppendorf tube formats. The results obtained with preparations in different formats showed no significant differences (t-test).

Conclusion

The use of the new Eppendorf Tubes 5.0 mL for processing of larger protein sample volumes allows considerable handling optimization of essential workflow steps compared to smaller tube formats. The new tubes can be easily employed for volumes between 1.0 mL and 5.0 mL; their conical shape assures thorough homogenization and spillage of samples may be avoided. The experiments have shown that all steps required for sample preparation can be easily adapted to the new tubes and that differences in temperature adjustment do not pose a problem during sample processing. Furthermore, neither quantitative nor qualitative deterioration of the proteins obtained could be detected.

** Manufacturer's safety notice: the specified operating temperature for Eppendorf Tubes 5.0 mL ranges from -86°C to +100°C. Work involving liquid nitrogen is carried out at user's own risk. For details please refer to the respective operating manual.*

Ordering information

Description	Order no. international	Order no. North America
Eppendorf Safe-Lock Tubes, 1.5 ml Colorless, Eppendorf Quality™	0030 120.086	022363204
Eppendorf Safe-Lock Tubes, 2.0 ml Colorless, Eppendorf Quality™	0030 120.094	022363352
Eppendorf Tubes® 5.0 mL Eppendorf Quality™, 200 tubes (2 bags of 100 ea.)	0030 119.401	0030119401
Eppendorf Tubes® 5.0 mL PCR clean, 200 tubes (2 bags of 100 ea.)	0030 119.460	0030119460
Eppendorf Tubes® 5.0 mL Sterile, 200 tubes (10 bags of 20 ea.)	0030 119.487	0030119487
Eppendorf Tubes® 5.0 mL Eppendorf Biopur®, 50 tubes (individually wrapped)	0030 119.479	0030119479
Eppendorf Tubes® 5.0 mL amber (light protection), Eppendorf Quality™, 200 tubes (2 bags of 100 ea.)	0030 119.452	0030119452
Eppendorf Protein LoBind Tubes 5.0 mL PCR clean, 100 tubes (2 bags of 50 ea.)	0030 108.302	0030108302
Eppendorf DNA LoBind Tubes 5.0 mL PCR clean, 200 tubes (4 bags of 50 ea.)	0030 108.310	0030108310
Starter Pack of Eppendorf Tubes® 5.0 mL PCR clean, 400 tubes (2 packages with 2 bags of 100 ea.), 2 racks (with 16 spaces), white, universal adapter for rotors with bore for 15 mL conical tubes (8 pcs.)	0030 119.380	0030119380

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