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Eppendorf Multipette®/ Repeater® E3x extends application diversity

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Executive Summary

An electronic dispenser compared to a manual dispenser offers higher flexibility due to the different modes and the broader range of possible dispensing volumes. The new Multipette/Repeater E3x even extends this flexibility by offering an additional mode, called "Aspirate and Dispense" (A/D). This mode allows the uptake of an unknown amount of liquid, followed by a subsequent delivery of

several dispensing steps of the same volume. Potential applications for this mode exist in protein biochemistry, molecular biology, cell biology and microbiology. This White Paper provides detailed information on several applications in the above fields where the usage of the mode A/D is advantageous.



Introduction

Liquid handling systems function according to two physical principles. Transfer of liquid either takes place via an air cushion or by positive displacement. The tips of positive displacement systems, e.g. Eppendorf Combitips advanced® have an integrated piston that is coupled with the piston of the instrument for liquid transfer. The liquid in the tip only reaches up to the integrated piston which is hermetically sealed by a sealing lip. This construction principle offers two main advantages. First, it ensures higher accuracy and precision when working with liquids whose physical properties differ from that of water. Second, the formation of aerosols is excluded which makes positive displacement instruments ideal for contamination-free work.

Eppendorf offers several liquid handling devices based on the positive displacement system. Among those the dispenser combines the advantage of a positive displacement system with the possibility of dispensing, meaning the aspiration

of liquid and the subsequent dispensing in equal/partial volumes. Eppendorf dispensers can be divided into two categories: manual and electronic devices. Manual dispensers like the Multipette/Repeater M4 are used for simple serial dispensing tasks where different modes or a high number of different dispensing volumes are not necessary.

Electronic dispensers, e.g. the Multipette/Repeater E3/E3x, offer a higher flexibility due to different modes. The new Multipette /Repeater E3x even extends this flexibility by offering the mode "Aspirate and Dispense" (A/D). This White Paper highlights possible applications in cell biology, microbiology, molecular biology and protein biochemistry.

Possible fields of applications

Basically mode A/D allows the uptake of an unknown amount of liquid and the subsequent dispensing of this volume in equal parts. The advantage of this new mode: it calculates the aspirated liquid volume and allows the user to define the volume to be dispensed. General application examples would be the uptake of a supernatant followed by the subsequent distribution to several reaction tubes, or if you want to calculate whether the small amount of liquid left in a supply bottle will be sufficient for your experiment. This mode can virtually be used for several applications in protein biochemistry, molecular biology, cell biology or microbiology. Examples of possible applications in each field will be discussed below.

Protein biochemistry

Classical protein biochemistry focuses on the understanding of the biological function of a protein, its amino acid composition, its structure and binding partners, the subcellular localization of the protein and finally its physiological role in the organism. In proteomics, a large-scale characterization of the proteome (whole set of proteins found in one cell line, tissue or organism at a defined biological situation and time point) of a sample is performed by using an integrated set of workflows. These workflows are used to characterize differences in protein expression between biological specimens. Important methods in protein research include protein purification, SDS-PAGE (poly acrylamide gel electrophoresis), HPLC, 2D gel electrophoresis, mass spectrometry, Western Blot and ELISA.

Protein extraction:

When using detergents for protein extraction the proteins are solubilized during extraction and are finally found in the supernatant. For supernatant uptake the Multipette/Repeater E3x in mode A/D can be used. The supernatant is aspirated, its volume calculated, and it could easily be distributed to an appropriate amount of reaction vessels.

Molecular biology

Molecular Biology is a branch of biology that deals with the molecular basis of biological processes. It concerns itself with the understanding of the DNA/RNA base sequence, the function of DNA/RNA sequences and the interaction between DNA, RNA and proteins. Furthermore, this field overlaps to a great extent with other areas of biology and chemistry, particularly biochemistry. Important methods in DNA/RNA research include DNA/RNA purification, polymerase chain reaction (PCR), sequencing etc.

DNA/RNA extraction:

One important method in DNA/RNA research is the extraction of DNA and RNA from different sample material e.g. cells, bacteria, plants. For DNA/RNA extraction different protocols are available. In several protocols, the DNA/RNA is found in the supernatant after extraction. Here mode A/D can be used to aspirate the supernatant and dispense it in equal volumes into several reaction vessels. So when aspirating the liquid the customer will immediately know for how many assays/aliquots the volume will last.

Cell Biology

Cell biology is a branch of biology that studies cells - the organelles they contain, their function, their physiological properties, their life cycle, the interactions with their environment etc. Cell biology basic research can be divided into several subfields e.g. the study of cell metabolism, the study of cell genetics and the underlying regulatory mechanisms, the study of cell compartment structures, the study of cell cycle, division and death and the study of cell communication and signaling.

Density gradient centrifugation:

One important technique in cell biology is the fractionation of cells into their components. This is often done using density gradient centrifugation. Here a homogenate of cells or tissue is pipetted on a density gradient. During centrifugation, the cell compartments will be enriched in their appropriate density zone. The density gradient is normally done using highly viscous solutions, e.g. sucrose. Thus the usage of a positive displacement instrument, like the Multipette/Repeater E3x in combination with Combitips advanced is advantageous. Furthermore, after completed centrifugation one has to separate the different layers (which contain the different cell compartments) of the density gradient. This can easily be done with the Multipette/Repeater E3x in A/D mode. It allows you to aspirate the liquid while counting the liquid volume and subsequently dispense the appropriate amount of liquid into several reaction vessels. The purified cell compartments are now ready to be used for different applications e.g. biochemical analysis of the protein composition.

Conditioned media:

To study cell metabolism or cell genetics etc. the cells are normally cultured under appropriate conditions in cell culture. To supply the cells with all nutrients and metabolites needed for cell growth and differentiation an appropriate medium is supplied. Sometimes the use of conditioned media is beneficial for cell lines that are difficult to cultivate. Conditioned medium is used medium harvested from cultured cells, which contains metabolites, growth factors and extracellular matrix proteins secreted into the medium by the cultured cells. The cell lines which require conditioned media are dependent on at least one of constituents found in the conditioned media. To remove the conditioned media from the cells the Multipette/Repeater E3x in A/D mode can be used. This mode allows the consequent removing of the media from several wells as a contamination between several wells is of no consequence here. Afterwards the aspirated volume is displayed and the customer will be able to set the dispensing volume as needed to cultivate the appropriate

amount of cultured cells.

Freezing of cells:

To store cells for an indefinite period of time they can be frozen in liquid nitrogen. Therefore the cells are resuspended in medium containing DMSO. To dispense the cell suspension into the prepared cryo vials again Multipette/Repeater E3x in A/D mode can be used.

Microbiology

Microbiology is the study of microorganisms, a large and diverse group of organisms (bacteria, archae, yeast, fungi, algae, protozoa, (viruses)) that exist as single cells or cell clusters.

Cultivation of microorganisms:

To study microorganisms these have to be cultivated under appropriate conditions. So, they are normally grown in deep-well plates, test tubes, tubes etc. using appropriate media. Here the Multipette/Repeater E3x in A/D mode can be used to aspirate media from storage flasks and dispense it in equal parts into several vessels, which are afterwards inoculated with the microorganisms.

Freezing of bacteria:

In case precious bacteria must be stored for several years, this is done at -70 °C in medium containing 15 % glycerol. These cryo stocks are normally prepared in replicates. Here the Multipette/Repeater E3x with its A/D mode could be used for adding 100 % glycerol into cryo vials and subsequent addition of the bacteria culture.

Ordering information Multipette® E3/E3x

Description	Order number
Multipette® E3 with charging adapter and 2 Combitips advanced® assortment packs	4987 000.010
Multipette® E3 with charging adapter, 2 Combitips advanced® assortment packs, and charging stand	4987 000.371
Multipette® E3x with charging adapter and 2 Combitips advanced® assortment packs	4987 000.029
Multipette® E3x with charging adapter, 2 Combitips advanced® assortment packs, and charging stand	4987 000.380
Charger stand for Multipette® stream/Multipette® Xstream/Multipette® E3 & E3x, for one device	4880 000.018
Charger Carousel for 4 Eppendorf Xplorer® and Eppendorf Xplorer® plus	4880 000.026
Multipette® charger shell for charger carousel Eppendorf Xplorer/ Eppendorf Xplorer plus	4880 000.026
Wallmount for Multipette® stream/Multipette® Xstream/Multipette® E3 & E3x	4986 604.001
Eppendorf TrackIT consisting of software and RFID reader	3903 000.014

Ordering information for Combitips advanced and accessories

Combitips advanced®	Color coding	Eppendorf Quality™ box of 100 pcs. (4 bags x 25 pcs.)	PCR clean*1 box of 100 pcs., 4 bags (zip-lock) x 25 pcs.	Eppendorf Biopur®*2 box of 100 pcs. (individually wrapped)
0.1 mL	□ White	0030 089.405	0030 089.766	0030 089.618
0.2 mL	■ Light blue	0030 089.413	0030 089.774	0030 089.626
0.5 mL	■ Purple	0030 089.421	0030 089.782	0030 089.634
1 mL	■ Yellow	0030 089.430	0030 089.790	0030 089.642
2.5 mL	■ Green	0030 089.448	0030 089.804	0030 089.650
5 mL	■ Blue	0030 089.456	0030 089.812	0030 089.669
10 mL	■ Orange	0030 089.464	0030 089.820	0030 089.677
25 mL*3	■ Red	0030 089.472	0030 089.839	0030 089.685
50 mL*3	■ Light gray	0030 089.480	0030 089.847	0030 089.693
Accessories				
25 mL adapter (1 pc.)	■ Red	0030 089.715		
25 mL adapter (7 pcs.)	■ Red			0030 089.731
50 mL adapter (1 pc.)	■ Light gray	0030 089.723		
50 mL adapter (7 pcs.)	■ Light gray			0030 089.740
Combitip Rack (for 8 Combitips advanced®, 0.1 mL–10 mL)		0030 089.758		
Combitips advanced Assortment pack (1 Combitip of each size, incl. adapters)		0030 089.936		

*1 PCR clean: batch tested and certified to be free of: human DNA, DNase, RNase, PCR inhibitors

*2 Biopur®: batch tested and certified to be sterile and free of: human and bacterial DNA, DNase, RNase, PCR inhibitors, ATP, Pyogen

*3 4 boxes of 25 pcs. each. Each box contains an adapter.

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