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APPLICATION NOTE No. 185

Automated Media Change in 24-well Plates for HeLa Cells Using the ep*Motion*[®] 5070 CB

Dagmar Bracht, Eppendorf Instrumente GmbH, Hamburg, Germany

Abstract

The Eppendorf ep*Motion*[®] 5070 CB enables trouble-free replacement of manual media change for HeLa cells with an automated protocol, thus demonstrating that semi-

automation of cell culture is feasible. Furthermore, foam formation of protein-rich medium is avoided and cells can adhere and grow properly.

Introduction

Cell culture systems are used in a variety of applications, making them indispensable in today's research work. For ethical as well as for economic reasons, the past few years have experienced a steady trend towards replacing animal experiments with cell culture systems, thus increasing the influence of cell culture on the research environment. Today, routine culturing of cells still requires numerous dedicated lab personnel. The ep*Motion* 5070 CB was conceived as an automated liquid handling system for specific use in the sterile environment of a laminar flow hood, therefore enabling the automation of many complex tasks inherent in cell culture. In this Application Note, the routine task of automated media change in 24-well plates with the ep*Motion* 5070 CB was examined for speed and ease of handling.

Materials and Methods

- > Eppendorf epMotion 5070 CB
- > Reservoir rack
- > Reservoirs, 100 mL
- > Height adapter, 85mm (optional)
- > 24-well cell culture plates
- $> epT.I.P.S^{\circledast}$ Motion, 1000 μL
- > HeLa cells
- > Medium (RPMI 1640) + 10% FCS (fetal calf serum)

During media changes old medium is removed from the cells, followed by replenishment with fresh, nutrient-rich medium. An additional wash step using PBS (phosphate buffered saline) is often undertaken, but this is not always necessary. Since it is not entirely possible to avoid a certain residual volume during automated handling of flat bottom plates, this wash step was not performed in order to prevent subsequent dilution of the fresh medium. It is possible to minimize residual volume by reducing the base tolerance [1] of the plate to a minimum.

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The fresh medium was provided in 100 ml reservoirs. Alternatively, 50 or 15 mL conical tubes may be used. However, in this case the automatic liquid level detection function is not available. An additional reservoir should be available to receive the used media. Both can be placed into a module rack [1], thus enabling the handling of two 24-well plates in one method. Since cell culture medium is protein rich and shows a tendency to foam, the Liquid Type "Protein" was chosen for all complex commands in an effort to avoid this effect.

top\Dagmar\Medienwechsel.lhs			
Tip 1000	MTP 24_1	empty	
Tip Rack	Rack		
A1	A2 1:2	PABK1	
7x100ml	MTP 24_2	empty	
Rack	Rack		
L _{B1}	L B2 1:2	PARK2	
		empty	
		L _{PABK3}	
WIN32 ready			
Prop. Info Start Exit			

Figure 1: Screen shot control panel. Loading of the ep*Motion* 5070 CB for the method "medium change" in 24-well plates.

The used medium is removed with the complex command "PoolOneDest" [1]. During this process foaming inside the tip can be caused by the FCS present in the medium if extensive concavity of the liquid surface is observed inside the well. Should this happen, a change in pipette tip will resolve the problem. Subsequently, the fresh medium is distributed via a further complex command "Reagent Transfer"[1]. In case a second medium change is planned during cultivation of the cells in this 24-well plate, it is recommended not to exceed a total volume of one milliliter.



Figure 2: Screen shot control panel.

List of commands necessary for the method "medium change" in 24-well plates on the ep*Motion* 5070 CB.

Results

The ep*Motion* 5070 CB enabled sterile medium change for HeLa cells in 24-well plates in an automated fashion.

The cells were viable, and cell adherence was not compromised in any way (Fig. 3).



Figure 3: Adherent HeLa cells in one well of a 24-well plate prior to (A) and following (B) automatic medium change using the ep*Motion* 5070 CB.

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Conclusions

The ep*Motion* 5070 CB is ideally suited for automatic medium change for adherent cells grown in 24-well plates, and therefore, for semi-automation of an important step in cell culture.

Literature

[1] Eppendorf manual for the epMotion 5070 CB.

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