

Application Note 12



Automated Media Change of GrowDex® Hydrogels using Blue®Washer Technology

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GrowDex-T & media layer on top





GrowDex*T after media removal



The Blue®Washer instrument of BlueCatBio uses centrifugation instead of aspiration to evacuate microtiterplates (media change, discarding supernatant, wash procedures, etc).

INTRODUCTION

High Throughput screening (HTS) with cell models requires automated handling of the cell culture microplates. The Blue®Washer uses contact-free centrifugation technology to remove liquids from 96, 384 and 1536 microwell plates without disposable tips. This environmentally friendly technology allows you to handle cellular, bead based, genomic, and ELISA assays contamination free with no residual volume, eliminating background and variability in assay data. The Blue®Washer also allows non-contact gentle evacuation of liquids from microplates having 3D cell culture embedded in hydrogel.

3D cell culture (spheroids, organoids) based on GrowDex® hydrogels is one of the most promising technologies to improve drug testing and related cell-based screenings. GrowDex hydrogels are made of nanofibrillar cellulose. As a shear thinning and temperature stable material, GrowDex is suitable for automated HTS protocols at room temperature. It is crucial that the supernatant (i.e., media) can be removed whilst keeping the hydrogel layer with spheroids intact to produce repeatable and standardized 3D assays with high throughput and precision. The growing market of 3D application demands reliable technology to perform media change in high throughput in an automated environment.

The aim of this study is to show automated removal of media in 96 and 384 well plates filled with GrowDex and GrowDex-T hydrogels. Parameters for the Blue®Washer have been found to work with a wide range of concentrations of the hydrogel (0.25% - 1%).



MATERIALS

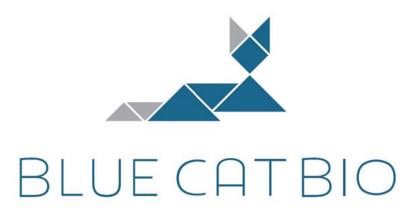
- Media: Gibco DMEM (1x) + GlutaMAXTM, (Cat No. Thermo Fisher Scientific 91965026)
- Microplate 96 (Cat No 92096, TPP Labtech, Switzerland); Microplate 384: Cat. No 781165, Greiner BioOne, Germany)
- GrowDex[®], 1.5% (Cat No 100 103 005, UPM Biomedicals, Finland)
- GrowDex®-T, 1.0% (Cat No 200 103 005, UPM Biomedicals, Finland)
- Blue Food Colour (RGM, Germany)
- Blue®Washer (BlueCatBio GmbH, Germany)
- Manual pipettors (Gilson)

METHODS

- 1. Stock GrowDex-T (1.0% w/v) was diluted with PBS to make a final working solution with a GrowDex-T concentration of 0.25%, 0.5% and 0.75%. Working example: for 1 ml of final working solution, add 200 µl of GrowDex-T to 800 µl of PBS and mix thoroughly by pipetting whilst avoiding bubbles. This will result in a 1 ml working solution of 0.2% GrowDex-T.
- 2. For 96 well plates, 80 μl of diluted hydrogel/well was transferred using a conventional manual pipettor. For 384 well plates the volume was 25 μl/well.
- 3. Hydrogels were stained with food colour to better visualize possible effects of centrifugal forces. For optimization of program parameters in the Blue®Washer (acceleration, decelaration, rpm, timing, etc.) the graphical user interphase (Blue®Flow, Fig. 1) was used in flat mode.



Figure 1. Blue®Flow software with implemented programs for media exchange of 96 well and 384 well plates in GrowDex hydrogel applications



RESULTS

In 384 well plates media change is possible with both, GrowDex and GrowDex-T. As an example, a GrowDex-T (0.5%) experiment is shown.



Figure 2. (a) 384 well plate with 25µl of 0.5% GrowDex-T per well, stained with food colour, (b) overlay of 80µl Media, (c) after removal of supernatant with Blue®Washer

In 96 well plates media change is possible with both, GrowDex and GrowDex-T. As an example, a GrowDex-T (0.5%) experiment is shown.



Figure 3. (a) 96 well plate with 100µl of 0.5% GrowDex-T per well, stained with food colour, (b) overlay of 150µl Media, and (c) after removal of supernatant with Blue®Washer.

CONCLUSION

The Blue®Washer enables automated media change for GrowDex 3D cell culture experiments in 96- as well as 384-well plates. This was successfully demonstrated for both, GrowDex and GrowDex-T hydrogels in the range of 0.25% - 1% working concentration. Easy, repeatable and fast removal of the culture medium from GrowDex microwell plates enables effective cell culture studies e.g. in HTS assays that are performed in drug discovery and development process.

ORDERING INFORMATION

Order GrowDex® hydrogels online at: biomedicalsshop.com



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