Application Note

Culturing embryonic stem cells using media filtered with Thermo Scientific Nalgene Rapid-Flow PES filter units.

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Key Words

Rapid-Flow™, PES filters, Stem Cells, Embryonic stem cells, Leukemia inhibitory factor, Pluripotency

Abstract

Eliminating contamination is important in cell culture, and sterile filtering of media is a standard practice to reduce the possibility of introducing contaminants to the culture system. However, due to the fastidious nature of embryonic stem cells (ESC), this common precautionary measure is often curtailed for fear of removing critical media components or adding deleterious compounds during the filtration process. Therefore, many stem cell researchers forego filtering certain media components such as the growth factors, leading to higher risk of contamination. The purpose of this study is to show that Thermo Scientific Nalgene Rapid-Flow™ PES filters do not remove critical media components or add harmful elements, and that ESC grown in media filtered through Rapid-Flow PES filters maintain normal growth and pluripotency.

Rapid-Flow Filters safely maintain sterility in stem cell cultures

Introduction

Maintaining pluripotent cells in culture represents a unique challenge for stem cell researchers. The culture system has to be closely controlled since any changes may easily trigger spontaneous differentiation or cell death. Growth factors, such as the leukemia inhibitory factor (LIF) normally expressed in the trophoectoderm of the developing embryo, are often added to the culture media to promote long-term maintenance and prevent unwanted differentiation of pluripotent stem cells. The cost of these media supplements is often high, but they are critical in maintaining the pluripotency of cells. Therefore, it is important to ensure that the growth factors are maintained in the growth media at the proper concentrations.

Growth media for cell culture is often sterile filtered in an effort to minimize the risk of contamination. Polyethersulfone (PES) membrane filters are used for this purpose due to the low protein binding and low extractable properties of the material. Filtering media for highly sensitive stem cells, however, introduces the possibility of removing important growth factors or adding compounds from the filter that may adversely affect the culture. To avoid this, many researchers add the most critical components (e.g. LIF) to the media after filtering. While this preserves the integrity of these components, it can be a vehicle for contamination.Here we show that filtering stem cell growth media using Thermo Scientific Nalgene Rapid-Flow PES filter units does not remove substantial amounts of LIF, nor does it add compounds that impair the growth of cells. Finally, filtering the complete stem cell growth media using Nalgene Rapid-Flow PES filter units does not adversely affect stem cells, allowing them to maintain their normal growth and pluripotency characteristics.

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Materials

Rapid-Flow PES Filter Units, Nalgene Cat. # 566-0020

Mouse Embryonic Stem Cells (mESC) PRX-6BN, Primogenix Cat. # SV30109.01

Mouse Embryonic Fibroblasts, GlobalStem Cat. # GSC-6001G

Mouse LIF Quantikine Assay, R&D Systems Cat. # MLF00

Anti-Sox2 antibody, Cell Signaling Cat. # 4900S

Anti-Oct-4 antibody, Millipore Cat. # MAB4419

ES Cell Characterization Kit with anti-SSEA-1 antibody, Millipore Cat. # SCR001

DAPI, Pierce Protein Research Cat. # 62248

Growth Media:

- AdvanceSTEM DMEM, HyClone Cat. # SH30824.01
- DMEM/High Glucose, HyClone Cat. # SH30022.01
- Fetal Bovine Serum, HyClone Cat. # SH3007003, SH3007003EH
- Penicillin-Streptomycin, Hyclone Cat. # SV30010
- SG-200, HyClone Cat. # SH30590.01
- Nucleosides, Millipore Cat. # ES-008-D
- 2-Mercaptoethanol, Sigma Cat. # M7522
- Leukemia Inhibitory Factor, Millipore Cat. # ESG1107
- HTF, LifeGlobal, Cat. # GMHT

DPBS, HyClone Cat # SH30028.02

0.1% Gelatin in Sterile Water, Millipore Cat. # ES-006-B

6-Well Multidish, Nunc Cat. # 140675

96-well optical bottom plate, Nunc Cat. # 164588

4-well IVF multidish, Nunc Cat. # 144444

Equipment

Mutidrop Combi plate dispenser, Thermo Scientific
Varioskan Flash spectrophotometer, Thermo Scientific

Methods

Mouse LIF ELISA

All reagents were prepared according to the instructions for use of the Mouse LIF ELISA Quantikine assay kit (R&D Systems). 1500 mL of Mouse ESC complete media was prepared and divided into three 500 mL aliquots. Each aliquot was filtered through ten 500 mL Rapid-Flow units of the same lot, and a sample of media was removed for testing after each filtration. Three different lots of the Rapid-Flow filters were used. New filter units were used for each filtration. Media samples were diluted 1:4 with PBS and tested according to LIF ELISA kit instructions. Plates were read on the Thermo Scientific Varioskan at 450 nm. A reference reading at 570 nm was subtracted from the test absorbance to eliminate background absorbance. The corrected absorbencies of the calibrator series were used to create a calibration curve with an R^2 value of 0.99. Linear regression analysis was then used to calculate the LIF concentration of the unknown samples.

Mouse Embryo Assay (MEA)

Mouse embryo assay testing was conducted using an independent third-party Quality Control testing laboratory. Briefly, three 50 mL batches of embryo culture media were filtered through Rapid-Flow filters from three different lots. Four 0.7 µL droplets of filtered media were placed in the wells of a Nunc IVF multidish, and 21 embryos were cultured in the media for each filter lot. 15 embryos were cultured separately in unfiltered media as controls. Embryos were monitored for 96 hours to determine their progression to blastocyst stage. Embryos were scored after this time, and a result greater than 70% of embryos progressing to blastocyst stage was considered a passed test.

Stem Cell Culture

Stem cells were obtained from an established pluripotent culture of mESC. Seven batches of mESC growth medium were prepared and sterile filtered prior to adding LIF. After adding LIF, 3 of the batches were filtered once using Rapid-Flow filter units of 3 different lots. An additional 3 batches of medium were filtered five times using Rapid-Flow filter units of 3 different lots. Each filtration was performed using a new filter unit. mESC were seeded on Nunc 6-well Multidishes with mitotically inactivated Mouse Embryonic Fibroblast (MEF) feeder cells previously seeded in MEF-specific growth media. MEF media was exchanged with test media upon mESC seeding. Cultures were maintained on 6-well dishes with MEFs through five passages. Cells were grown two days between passages and media was changed on each day between passages. All feeding and passaging was performed with the proper medium batch corresponding to the test condition. Bright field photographs were taken in the fifth passage of cells on 6-well Multidishes. Also on the fifth passage, an additional Nunc 96-well optical bottom plate was cultured with mESC for approximately thirty hours before immunostaining of pluripotency markers.

Immunocytochemistry

Immunocytochemistry was performed in a Nunc 96-well optical bottom plate with a MEF feeder layer. Cells were fixed with 4% paraformaldehyde solution for 15 minutes. Cells were rinsed three times with DPBS and were blocked with 10% goat serum diluted in DPBST (DPBS with 0.1% TX-100) for 30 minutes at room temperature. Primary antibodies, SSEA-1, Oct-4, and Sox2 were diluted 1:400 in blocking solution and were added for an overnight incubation at 2-8°C. The cells were rinsed with DPBST three times and the appropriate secondary antibody (Alexa-488-gAM IgG, Alexa 488-gAR IgG, and Alexa 555-gAM IgM) diluted 1:1000 in blocking solution were incubated for one hour at room temperature. Cells were rinsed once with DPBST and DAPI diluted 1:1000 in DPBS was incubated for 5 minutes at room temperature. Cells were rinsed two times with DPBST followed by two washes with DPBS. The cells were stored in the second DPBS wash in the dark at 2-8°C until image analysis was completed. Bright field and fluorescent images were visualized with a Zeiss Axiovert 200 microscope and images were acquired with a Photometrics CoolSNAP ES^2 camera. Image analysis was completed with AxioVision software.

Results and discussion

Nalgene Rapid-Flow PES filters do not diminish LIF content in the mESC growth media

The amount of LIF in mESC complete media was measured following successive filtration. ELISA results indicate that one time filtration did not appreciably affect the LIF concentration (Figure 1). Even over the course of ten filtrations, no more than 5% of LIF in the growth media is lost to filtration (Figure 1).

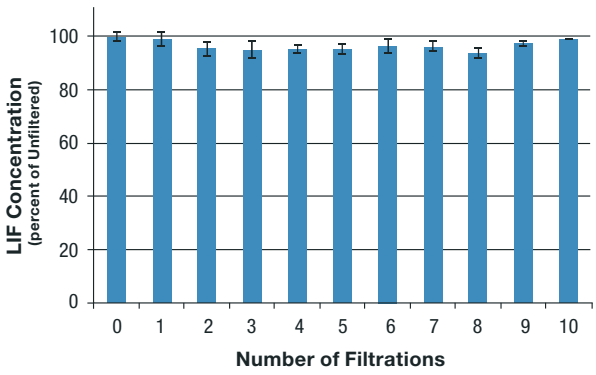

Figure 1. The retention of LIF in the complete mESC growth media following multiple filtrations using Nalgene Rapid-Flow PES filters. Filtration did not appreciably affect LIF concentration.

Nalgene Rapid-Flow PES filters do not add deleterious compounds to the filtered media

The highly sensitive Mouse Embryo Assay was conducted to assess potential harmful additions to the culture media from the filtration process. For two out of three tested filter lots, 95% of mouse embryos tested grew to blastocyst stage by 96 hours. For the remaining filter lot, 100% of the embryos progressed to blastocyst stage after 96 hours. These results indicate that filtering of media using Nalgene Rapid Flow PES filters does not add any deleterious compounds to the media that will affect cell growth

Media filtered with Nalgene Rapid-Flow PES filters support mESC growth and pluripotency

Mouse embryonic stem cells were cultured on feeder cells through five passages using filtered medium. The mESC proliferated well throughout the span of the culture and displayed normal ESC morphology (figure 2, bright field). The mESC pluripotency was evaluated by immunofluorescence staining of SSEA-1, OCT-4, and Sox2 markers (figure 2). In both brightfield and immunostained conditions, mESC cultured in filtered media yield comparable results as that of the control. These results indicate that mESC pluripotency was maintained throughout the time in culture with filtered medium containing LIF.

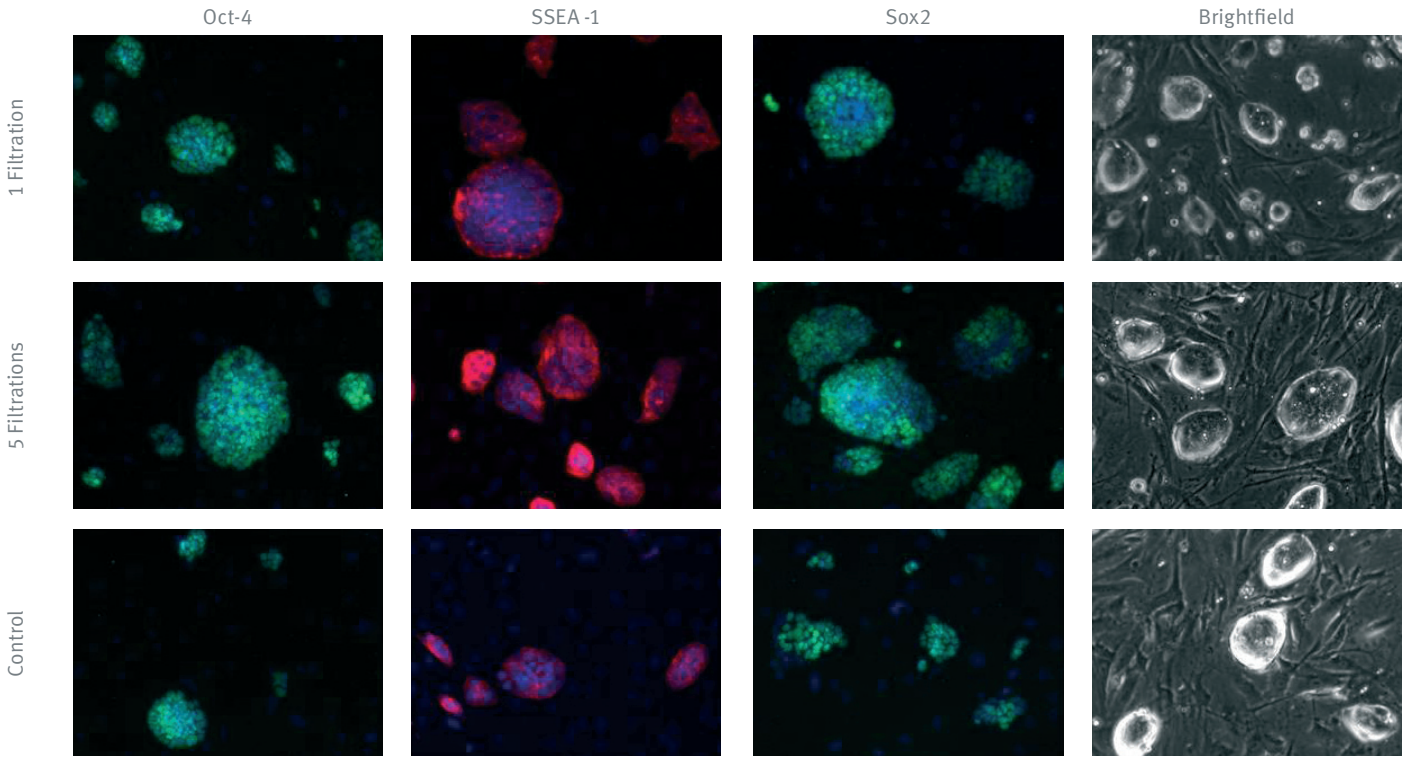

Figure 2. Immunofluorescence staining of pluripotent markers on mESC after being cultured for 5 passages in filtered media. 1 Filtration = media filtered once after formulation; 5 Filtrations = media filtered 5 times after formulation; and Control = media not filtered. Cells were counterstained with DAPI nuclear stain. mESC cultured in filtered media yielded comparable results to unfiltered controls.

Discussion

Multiple media filtrations were conducted during this study in order to magnify any possible effect the filtration may have. For each successive filtration a new filter unit was used so that any compound that may have been removed by filtration would be truly eliminated, and any compound that may have been added by the filter would continue to be added as new filters were used. Such stringent conditions are unlikely under normal application usage, however such a study ensures that any minimal effect that filtering may have becomes apparent during experimentation.

Despite the extreme conditions posed by multiple media filtrations, embryonic stem cells continued to grow well and maintain pluripotency throughout the passages. These results indicate Nalgene Rapid-Flow PES filters are a safe solution for maintaining sterility in stem cell cultures.

Conclusion

- Nalgene Rapid-Flow filter units do not retain nor add components to the filtered media.
- Sterile filtration of complete ESC growth media using Nalgene Rapid-Flow filter units does not adversely affect growth or pluripotency of the stem cells.
- Nalgene Rapid-Flow PES filter units are a safe solution for maintaining sterility in stem cell cultures.

Additional Resources

- www.thermoscientific.com/rapidflow
- Nalgene Rapid-flow brochure (#BRLSPRF)
- "Flow rate and throughput of cell culture media and serum using Thermo Scientific Nalgene Rapid-Flow filters with 0.2 micron PES membranes" (Application note #ANLSP02RF).
- "Flow rate and throughput of Thermo Scientific Nalgene 0.1 micron Rapid-Flow PES filter units with cell culture media" (Application note #ANLSPFILT01PES).

Ordering Information

Polyethersulfone (PES) is the ultimate tissue culture membrane. It's fast and clean. PES is low protein binding, so there is less chance of removing critical protein from your media. It is hydrophilic, so no external wetting agents or surfactants are needed, resulting in lowextractables. And it is fast, so you spend less time waiting.

Nalgene Filters with PES Membrane

Capacity ml	Pore Size µm	Membr. Diam. mm	Fits Bottle Neck Size	Number Per Case	Catalog Number
PES Filter Units					
50	0.2	50	Tube	12	564-0020
150	0.1	50	—	12	565-0010
150	0.2	50	—	12	565-0020
150	0.45	50	—	12	165-0045
250	0.1	50	—	12	568-0010
250	0.2	50	—	12	568-0020
250	0.45	50	—	12	168-0045
500	0.1	75	—	12	566-0010
500	0.2	75	—	12	566-0020
500	0.45	75	—	12	166-0045
500	0.2	90	—	12	569-0020
500	0.45	90	—	12	169-0045
1000	0.1	90	—	12	567-0010
1000	0.2	90	—	12	567-0020
1000	0.45	90	—	12	167-0045
PES Bottle Top Filters					
150	0.2	50	33	12	596-3320
150	0.45	50	33	12	296-3345
150	0.2	50	45	12	596-4520
150	0.45	50	45	12	296-4545
500	0.2	75	33	12	595-3320
500	0.45	75	33	12	295-3345
500	0.2	75	45	12	595-4520
500	0.45	75	45	12	295-4545
1000	0.2	90	33	12	597-3320
1000	0.2	90	45	12	597-4520

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