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Application Note

T cell expansion and viability using Stericup®-filtered media

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Introduction

T cells require contamination-free cell culture media that contain cytokines, serum, and supplements optimized for cell viability, expansion, or bioprocessing. Filter sterilization is the optimal method for cell culture media preparation. The right filter membrane will remove particulates and contaminants (e.g., microorganisms, bacteria, etc.) while preserving the integrity of key heat-labile growth factors and supplements required for T cell function. Our Stericup® Quick Release sterile vacuum filtration systems minimize protein loss and denaturation in cell culture media preparation. Our Stericup® E and Steritop® E sustainable filtration devices provide the added benefit of reducing plastic waste while conserving storage space in your cell culture lab.

In this study, we examined the impact of filtration of hematopoietic cell culture media containing vital T cell factors and other media components after repeated filtration using Stericup® sterile filtration devices. We evaluated T cell growth, morphology, marker expression, and cytokine secretion using filtered media. Filtration of cell culture media using Stericup® devices supports viability and worry-free expansion of T cells, aiding in the production of T cell and CAR-T cell therapies.

Background

Researchers use great care to prevent contamination and maintain sterility during the culturing of T cells for basic research, drug discovery, therapeutic development, and manufacturing. All cell culture components and solutions must be sterilized, starting with the culture medium and any additives. Sterilization by autoclaving methods is time-consuming and limited when there is a presence of heat-sensitive reagents such as cytokines and other growth factors in the media. Previous studies found that filtration of CD3+, a ubiquitous precursor for progenitor T cells, had a negligible effect on the naïve, inactivated state of T cells.[1] Another study found that, for each filtration of cell culture media, the same ratio of CD4+/CD8+ markers was present on immune cells in culture (indicating helper and cytotoxic T cell phenotype).[2] These studies suggest media filtration does not impact phenotype, viability, or potential T cell autoregulation.

Interleukin-2 (IL-2) is a crucial immunomodulatory cytokine added to culture media for stimulation of T cell growth and is innately produced by CD4+ T cells, effecting the subsequent killing of tumor cells.[3] The integrity of the culture medium after filtration is critical for T cell culturing, phenotype, and expression.

Filtration using Stericup® sterile vacuum filtration devices containing 0.22 µm filter membranes provides a convenient, rapid, and effective method for cell culture media preparation (**Figure 1**). These devices are available with different membrane types for cell culture applications. When media contain serum, polyethersulfone (PES) filter membranes are generally preferred due to their faster flow speed and higher throughput. Durapore® polyvinylidene fluoride (PVDF) is used when lower protein binding is needed or desired. Both membranes remove particulates and contaminants such as microorganisms, bacteria, and yeast while preserving key growth factors and supplements necessary for T cells in culture.

Figure 1. Stericup® Quick Release Vacuum Filtration Systems (left) and Stericup® E Sustainable Vacuum Filtration Systems (right) for sterile filtration of cell culture media, buffer, and reagents.

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Methods

Media preparation

Formulated T cell medium was prepared using Hematopoietic Cell Medium (Lonza Bioscience #BEBP02-054Q), 2% Human Serum (Cat. No. H4522), and 50 IU/mL IL-2 (Cat. No. GF333) prepared in 100 mM acetic acid (Cat. No. 695092) with 1 mg/mL BSA (Cat. No. 126575). Subsequently, the medium was vacuum-filtered at 15" Hg according to instructions using Stericup® E-GP filters with 0.22 µm Express® PLUS PES membranes (Cat. No. SEGPU0538) or Stericup® Quick Release-GV filters with low protein binding 0.22 µm Durapore® PVDF membranes (Cat. No. S2GVU05RE). Media were filtered one time (typical use), five times, or 10 times. For each filtration step, a new filter unit was used. The final filtered media were used for T cell culturing and analysis over six days (**Figure 2**).

Media analysis

When media formulation and filtration were complete, the media preparation was analyzed for basic metabolite, gas, and osmolarity composition utilizing a Nova Biomedical BioProfile® FLEX2 device.

Culturing and cell measurements

2 x 10^6 donor T cells were placed in a T25 flask with filtered culture medium. T cells were cultured for six days without media exchange. On days 3, 5, and 6, the cell suspension was removed from the flask. Cell counting and diameter measurements were carried out using Scepter™ 3.0 Handheld Automated Cell Counter with 40 µm sensors (Cat. No. PHCC340KIT, ~100 µL/sample) and flow cytometry (NovoCyte® Flow Cytometer, Agilent Technologies). Cell concentration, cell viability, and growth curves were compared (**Figure 3**).

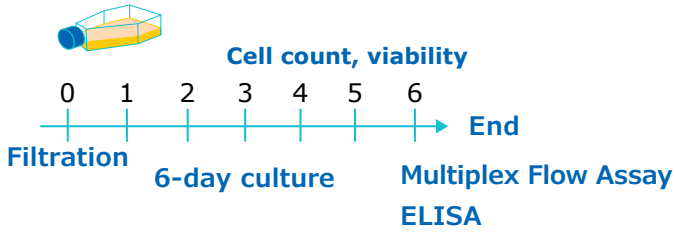
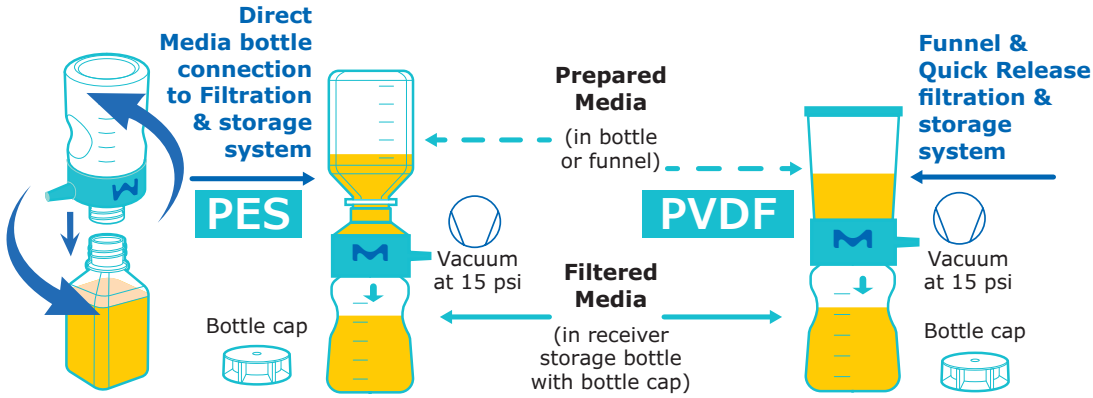


Figure 3. Outline of experiments for T cell culturing using T25 flasks.

Multiplex flow assays and ELISA

On Day 6, the cultured T cells were assessed for standard T cell markers (CD3+, CD4+, and CD8+) using a multiplex flow assay via flow cytometry (NovoCyte® Flow Cytometer, Agilent Technologies). IL-2 was quantitated using a human IL-2 ELISA kit (Cat. No. RAB0286) and prepared IL-2 standards.



Number of Filtrations	Total Filter Surface Area Used (cm^2)
1x	40
5x	200
10x	400

Figure 2. Schematic of media preparation used for T cell culturing. Single-use (1x) Stericup® filtration systems contain 40 cm^2 of membrane material.

Results

Filter membrane materials have different properties such as flow rate, protein retention, and levels of extractables. We determined the flow rates of Stericup® devices containing PES and PVDF filter membranes. As expected, faster flow was observed using Stericup® devices with PES Express® PLUS membrane (500 mL of prepared media) as compared to Durapore™ PVDF membrane (200 mL of prepared media). Subsequent filtrations had faster flow rates (**Figure 4**).

The composition of the filtered media was also assessed using a cell culture analyzer. No substantial metabolite, gas, or osmolarity differences were observed from any of the T cell media preparations post-filtration using Stericup® PES or PVDF devices (Table 1).

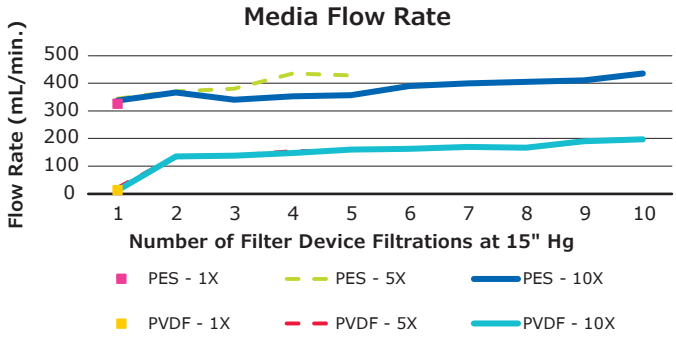


Figure 4. Media filtration flow rate comparison using single Stericup® PES and PVDF devices.

Filtered Media	Glutamine (mmol/L)	Glutamate (mmol/L)	Glucose (g/L)	Lactate (g/L)	NH^{4+} (mmol/L)	Na^{+} (mmol/L)	K^{+} (mmol/L)	Ca^{2+} (mmol/L)	pH	PO_2 (mm Hg)	PCO_2 (mm Hg)	Osmolarity (mOsm/kg)
PES - 1X	3.25	0.71	7.37	0	0.59	134.2	4.6	1.20	7.4	196	41.5	342
PES - 5X	3.23	0.85	7.36	0.01	0.57	135.6	4.6	1.21	7.5	198	32.4	339
PES - 10X	3.25	0.78	7.39	0	0.57	136.2	4.7	1.21	7.6	198	26.1	341
PVDF - 1X	3.26	0.81	7.40	0	0.55	136.5	4.7	1.22	7.53	200	30.9	345
PVDF - 5X	3.26	0.82	7.40	0.01	0.56	137.5	4.7	1.22	7.60	199	26.3	343
PVDF - 10X	3.26	0.86	7.40	0.01	0.54	136.5	4.7	1.21	7.75	198	19.2	344

Table 1. Metabolites, osmolarity, and gas content across filtered media.

T cells were cultured in hemopoietic media filtered using Stericup® E PES or Stericup® Quick Release PVDF devices. Filtration of culture media with Stericup® devices had no adverse impact on the T cell health, T cell phenotype, viability, cell concentration, or cell diameter during a six-day cycle. T cell phenotype marker expression (CD3+/CD4+/CD8+) and viability remained consistent for six days after filtration (**Figures 5–6**).

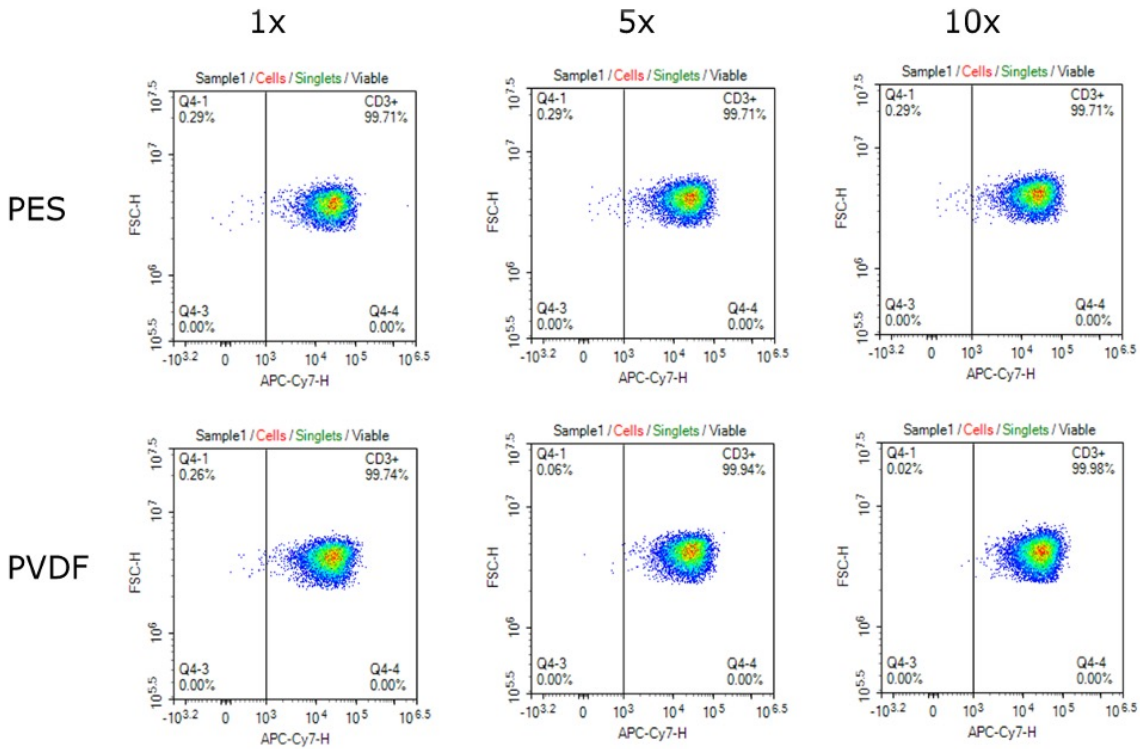


Figure 5. CD3+ phenotype distribution using T cell cultured in filtered media, by membrane type.

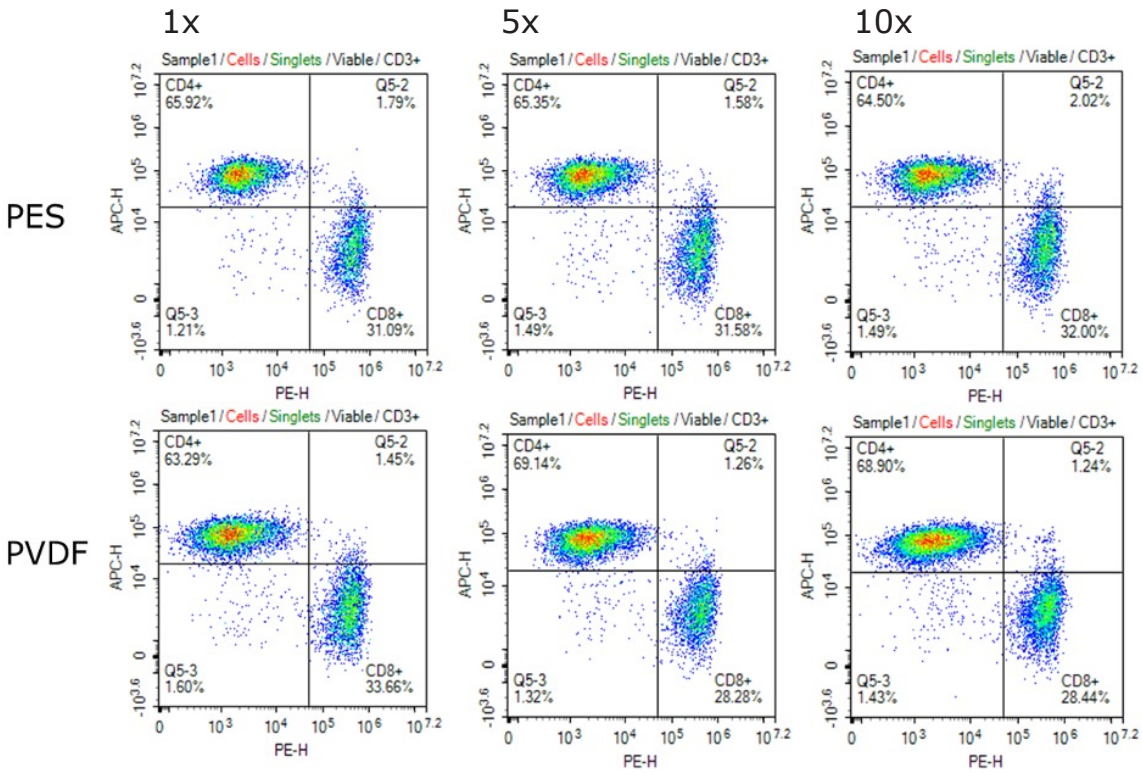


Figure 6. CD4+/CD8+ multiplex flow assay for T cells cultured in each filtered medium.

Viability, diameter, and concentration were measured throughout the cycle. T cell viability analysis showed a consistent pattern in the number of viable T cells across different media preparations. A sharp increase in the number of cells was observed post-thaw, and viability remained at ~90% through day 6 (**Figures 7-8**).

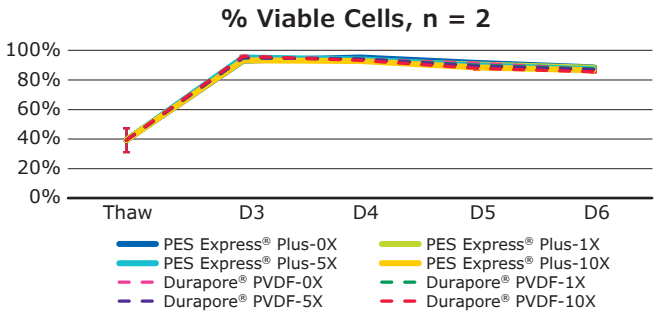


Figure 7. Percent of viable T cells for each filtered medium on different days.

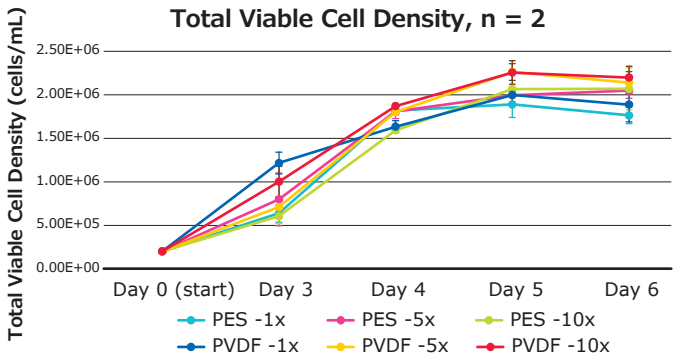


Figure 8. Total viable cell density for T cells cultured in each filtered medium on different days.

The Scepter™ 3.0 counter is a handheld device that can count and size cells using the Coulter impedance principle (**Figure 9**). Scepter™ histograms displaying cell size distribution showed comparable average diameter and concentration for T cells grown in all filtered media samples (**Figure 10**).

Figure 9. Scepter™ 3.0 handheld cell counter.

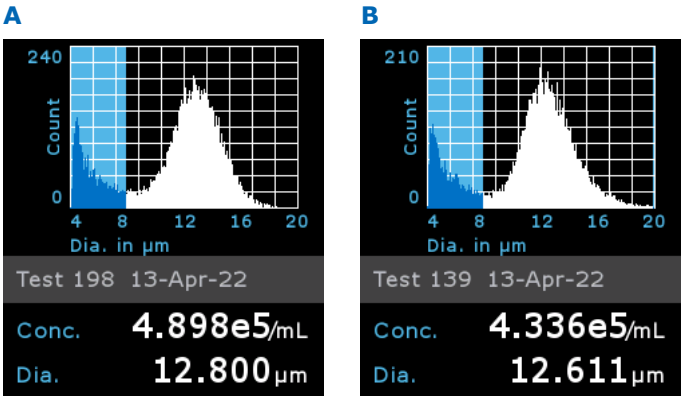


Figure 10. Representative Scepter™ 3.0 histograms using 5x filtered media with A) PES filter membrane and B) PVDF membrane on Day 5 (gated population between 8 and 20 µm).

There was no significant difference in average T cell diameter. Overall average diameters were 12.70 ±0.44 µm for PES and 12.73 ±0.31 µm for PVDF membranes, respectively (**Figure 11**). T cell concentrations remained consistent between filtrations when analyzed using Scepter™ 3.0 cell counting and flow cytometry measurements (**Figure 12**).

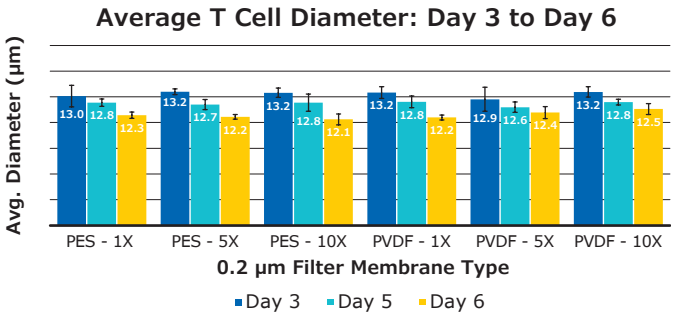


Figure 11. Average T cell diameter using six replicates per media sample. Measurements were taken on Day 3, 5, and 6 using the Scepter™ 3.0 handheld cell counter.

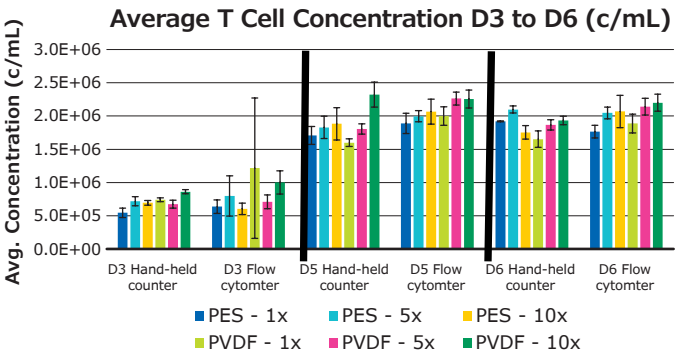


Figure 12. Average T cell concentration using six replicates for each filtered media sample. Measurements were performed on Day 3, 5, and 6. Results were determined using both Scepter™ 3.0 handheld cell counter and flow cytometry data.

Finally, IL-2 cytokine concentration was measured in T cell culture media. IL-2 was supplemented into the culture media prior to filtration. IL-2 is also secreted by T cells. Generally, IL-2 was consumed throughout the culturing time and no significant differences in concentration were observed. The added IL-2 in the media was not lost or adsorbed during filtering (**Figure 13**).

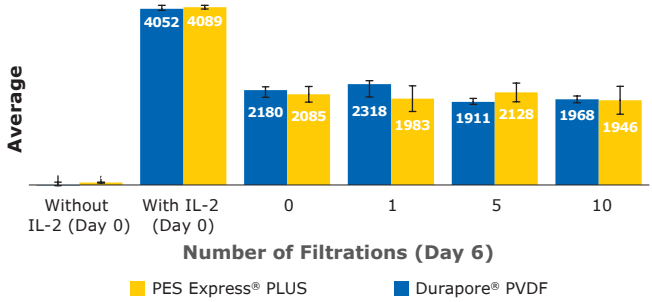


Figure 13. IL-2 concentration measured in T cell culture media at Day 6.

These results highlight the ability to culture T cells or CAR-T cells in filtered media without any major noticeable effect on phenotype (CD3+/CD4+/CD8+), cell size, viability, or levels of secreted cytokines such as IL-2. The different T cell phenotype markers were consistently expressed (CD3+ at ≥ 99% and CD4+/CD8+ at ≈65%/≈35%) regardless of filtration membrane and filtration frequency after initial cell thaw and at culture day 5. The IL-2 concentration in all filtered media remained comparable (≈ 2 ng/mL) through six days of cell culture. T cell density, concentration, and diameter also showed no consequential deviation in each filtered media sample across different cell culture periods. Our data highlight the ability to expand T cells in filtered media using Stericup® devices containing PES Express® PLUS and Durapore™ PVDF membranes without a noticeable effect on T cell viability, phenotype, or function. Stericup® sterile vacuum filtration units can be used in preparation T cell culture media for development and manufacturing of cell-based therapies and other applications.

References

1. Mousset, C.M., Hobo, W., Woestenenk, R., Preijers, F., Dolstra, H. and van der Waart, A.B., 2019. Comprehensive phenotyping of T cells using flow cytometry. Cytometry Part A, 95(6): 647-654.
2. Laidlaw, B., Craft, J. & Kaech, S., 2016. The multifaceted role of CD4+ T cells in CD8+ T cell memory. Nat Rev Immunol 16: 102-111.
3. De Berardinis, P., 1991. "T cell subsets and their lymphokines." Annali dell'Istituto superiore di sanita 27(1): 41-9.

Bench-scale Filters

Stericup® E and Steritop® E Eco-Friendly Filter Units—Greener Solution

Description	Membrane/Application	Pore Size (µm)	Receiver Bottle (mL)	Thread Size (mm)	Qty/Pk	Cat. No.
Stericup® E-GP Sterile Vacuum Filtration System	Millipore Express® PLUS (PES)/fast filtration of tissue culture media and buffers	0.22	500	38	12	SEGPU0538
			500	45	12	SEGPU0545
			1000	38	12	SEGPU1138
			1000	45	12	SEGPU1145
Steritop® E-GP Sterile Vacuum Filtration System	Millipore Express® PLUS (PES)/fast filtration of tissue culture media and buffers	0.22	All Volumes	38	12	SEGPT0038
				45	12	SEGPT0045

Stericup® E & Steritop® E Filter Systems

The new 'E' (eco-friendly) additions to the Stericup® family eliminate the plastic filter funnel entirely by threading directly onto the media bottle. Stericup® E and Steritop® E filter devices reduce environmental impact by cutting down on:

- **Disposable plastic**
- **Hazardous waste**
- **Lab storage space requirements**

Stericup® Filter Units

Stericup® Filtration Systems combine a filter unit with a receiver flask and cap for processing and storage.

Description	Membrane/Application	Pore Size (µm)	Funnel Capacity (mL)	Receiver Bottle (mL)	Qty/Pk	Cat. No.
Stericup®-GP Quick Release Filter Units	Millipore Express® PLUS (PES)/fast filtration of tissue culture media and buffers	0.22	150	150	12	S2GPU01RE
			250	250	12	S2GPU02RE
			500	500	12	S2GPU05RE
			500	1000	12	S2GPU10RE
			1000	1000	12	S2GPU11RE
Stericup®-HV Quick Release Filter Units	Durapore®(PVDF)/filtration of high value biomolecules, lowest protein binding	0.45	150	150	12	S2HVU01RE
			250	250	12	S2HVU02RE
			500	500	12	S2HVU05RE
			1000	1000	12	S2HVU11RE
Stericup®-VP Quick Release Filter Units	Millipore Express® (PES)/removal of mycoplasma*	0.1	250	250	12	S2VPU02RE
			1000	1000	12	S2VPU11RE
Stericup®-GV Quick Release Filter Units	Durapore® (PVDF)/filtration of high value biomolecules, lowest protein binding	0.22	150	150	12	S2GVU01RE
			250	250	12	S2GVU02RE
			500	500	12	S2GVU05RE
			500	1000	12	S2GVU10RE
			1000	1000	12	S2GVU11RE

* 0.10 µm pore size is designed to enhance maximum filtration of tissue culture media but it is not a guarantee of complete mycoplasma removal

Steritop® Filter Units

Description	Membrane/Application	Pore Size (µm)	Funnel Capacity (mL)	Thread Size (mm)	Qty/Pk	Cat No.
Steritop® QR Quick Release Filter Units	Millipore Express® PLUS (PES)/fast filtration of tissue culture media and buffers	0.22	150	45	12	S2GPT01RE
			250	45	12	S2GPT02RE
			500	45	12	S2GPT05RE
			1000	45	12	S2GPT10RE
Steritop®-GP Quick Release Filter Units	Millipore Express® PLUS (PES)/filtration of high value biomolecules, lowest protein binding	0.22	150	33	12	SCGPS01RE
			250	33	12	SCGPS02RE
			500	33	12	SCGPS05RE
Steritop®-GV Quick Release Filter Units	Durapore® (PVDF)/filtration of high value biomolecules, lowest protein binding	0.22	500	45	12	S2GVT05RE
Steritop®-VP Quick Release Filter Units	Millipore Express® (PES)/removal or mycoplasma*	0.1	1000	45	12	S2VPT10RE
Click Seal Receiver Bottles and Caps			250	45	12	S200B02RE
			500	45	12	S200B05RE
			1000	45	12	S200B10RE

* 0.10 µm pore size is designed to enhance maximum filtration of tissue culture media but it is not a guarantee of complete mycoplasma removal

Additional Products

Description	Cat. No.
IL-2, Human (Recombinant, Animal Free)	GF333
Human IL-2 ELISA Kit	RAB0286
Human Serum	H4522
EmbryoMax® 1X Dulbecco's Phosphate Buffered Saline w/o Ca++ & Mg++.	BSS-1006
Acetic acid	695092
Albumin, Bovine Serum, Fraction V (fatty acid-free)	126575
Scepter™ 3.0 Handheld Automated Cell Counter with 40 µm Sensors	PHCC340KIT
Millipore® Vacuum Filtering Side-Arm Flask, 1 L, Threaded Side-Arm	XX1014705
Tubing, silicone 4.8 mm ($^{3}/_{16}$ in) ID x 140 cm (4.5 ft)	XX7100004
No. 8 perforated stopper, silicon	XX1004708
Millex® FG, 0.20 µm, hydrophobic PTFE (polytetrafluoroethylene), 50 mm	SLFG05000
Chemical Duty Pump, 115 V/60 Hz	WP6111560

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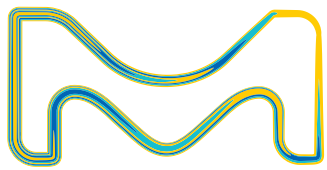

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