

Materials List for

Large-Scale Cell Production Based on GMP-Grade Dissolvable Porous Microcarriers

Yuyang Chen*¹, Huanye Xu*², Yuanyuan Zhang*², Leilei Guo², Miaomiao Lan², Yanping Yang², Wei Liu², Xiaojun Yan², Yanan Du¹

Corresponding Authors		Citation	
Xiaojun Yan yanxiaojun@cytoniche.com	Yanan Du duyanan@tsinghua.edu.cn	Chen, Y., Xu, H., Zhang, Y., Guo, L., Lan, M., Yang, Y., Liu, W., Yan, X., Du, Y. Large-Sca Cell Production Based on GMP-Grade Dissolvable Porous Microcarriers. <i>J. Vis. Exp.</i> (19 e65469, doi:10.3791/65469 (2023).	
Date Published		DOI	URL
July 7, 2023		10.3791/65469	jove.com/video/65469

Materials

Name	Company	Catalog Number	Comments
0.25% trypsin EDTA	BasalMedia	S310JV	Used for 2D cell harvest.
3D FloTrix Digest	CytoNiche Biotech	R001-500	This is a reagent that specifically dissolves 3D TableTrix microcarriers.
3D FloTrix MSC Serum Free Medium	CytoNiche Biotech	RMZ112	This is a serum-free,animal-free medium for mesenchymal stem cell expansion and maintenance in 2D planar culture as well as 3D culture on 3D TableTrix microcarriers.
3D FloTrix Single-Use Filtration Module	CytoNiche Biotech	R020-00-10	This module contains 0.22 µm capsule filters used for filtration of culture medium and digest solution.
3D FloTrix Single-Use Storage Bag (10 L)	CytoNiche Biotech	R020-00-03	Used as feed bag for 5 L bioreactor.
3D FloTrix Single-Use Storage Bag (3 L)	CytoNiche Biotech	R020-00-01	Used as cell seeding or transfer bags.
3D FloTrix Single-Use Storage Bag (50 L)	CytoNiche Biotech	R020-00-04	Used as feed bag for 15 L bioreactor.
3D FloTrix vivaPACK Disposable Fill&Finish Consumable Kit	CytoNiche Biotech	PACK-01-01	This is a standard kit adapted to 3D vivaPACK fill and finish system.
3D FloTrix vivaPACK fill and finish system for cells	CytoNiche Biotech	vivaPACK	This system is a closed liquid handling device, with automated mixing and gas exhausting functions. Cells resuspended in cryopreservation buffer can be rapidly and evenly aliquoted into 20 bags per batch.
3D FloTrix vivaPREP PLUS cell processing system	CytoNiche Biotech	vivaPREP PLUS	This system is a continuous flow centrifuge-based device. Cells can be concentrated, washed, and resuspended under completely closed procedures.
3D FloTrix vivaPREP PLUS Disposable Cell Processing Kit	CytoNiche Biotech	PREP-PLUS-00	This is a standard kit adapted to 3D vivaPREP PLUS cell processing.
3D FloTrix vivaSPIN bioreactor 15 L	CytoNiche Biotech	FTVS15	This bioreactor product employs a controller, a 15 L glass stirred-tank

¹Department of Biomedical Engineering, School of Medicine, Tsinghua University ²Beijing CytoNiche Biotechnology Co. Ltd.

^{*}These authors contributed equally



			vessel, and assessories. A special perfusion tube is available.
3D FloTrix vivaSPIN bioreactor 5 L	CytoNiche Biotech	FTVS05	This bioreactor product employs a controller, a 5 L glass stirred-tank vessel, and assessories. A special perfusion tube is available.
3D FloTrix vivaSPIN Closed System Consumable Pack (10/15 L)	CytoNiche Biotech	R020-10-10	This is a standard tubing kit adapted to 3D vivaSPIN bioreactor 15 L, containing sampling bags.
3D FloTrix vivaSPIN Closed System Consumable Pack (2/5 L)	CytoNiche Biotech	R020-05-10	This is a standard tubing kit adapted to 3D vivaSPIN bioreactor 5 L, containing sampling bags.
3D TableTrix microcarriers G02	CytoNiche Biotech	G02-10-10g	These porous and degradable microcarriers are suitable for HEK293T cell culture. They come pre-sterilized in 10g/bottle with C-Flex tubings for welding to tubes on bioreactors.
3D TableTrix microcarriers V01	CytoNiche Biotech	V01-100-10g	These porous and degradable microcarriers are suitable for adherent cell culture, they come as non-sterilized microcarriers that need to be autoclaved in PBS before use. They are especially suitable for vaccine production.
3D TableTrix microcarriers W01	CytoNiche Biotech	W01-10-10g (single-use packaging); W01-200 (tablets)	These porous and degradable microcarriers are suitable for adherent cell culture, especially for cells that need to be harvested as end products. They come pre-sterilized in 10g/bottle with C-Flex tubings for welding to tubes on bioreactors. The product has obtained 2 qualifications for pharmaceutical excipients from CDE, with the registration numbers of [F20200000496; F2021000003]. It has also received DMF qualification for pharmaceutical excipients from FDA, with the registration number of [DMF:35481]
APC anti-human CD45 Antibody	BioLegend	368512	Used in flow cytometry for MSC identity assessment
Calcein-AM/PI Double Staining Kit	Dojindo	C542	Calcein-AM/PI Double Staining Kit is utilized for simultaneous fluorescence staining of viable and dead cells. This kit contains Calcein-AM and Propidium lodide (PI) solutions, which stain viable and dead cells, respectively.
Cap for EZ Top Container Closures for NALGENE-containers (500mL)	Saint-Gobain	CAP-38	Brands and catalogue numbers are only for example, similar products are available from various suppliers and as long as they have the same functionality, items could be substituted with other brands.
C-Flex Tubing, Formulation 374 (0.25 in x 0.44 in)	Saint-Gobain	374-250-3	Used for tube welding and disconnection.
CryoMACS Freezing Bag 50	Miltenyi Biotec	200-074-400	Used for expanding the 3D FloTrix vivaPACK Disposable Fill&Finish Consumable Kit.
Dimethyl Sulfate (DMSO)	Sigma	D2650-100mL	Used for preparation of cryopreservation solution.
Dulbecco's Modified Eagle Medium (DMEM)	BasalMedia	L120KJ	Used for cultivation of HEK293T and Vero cells.



DURAN Original GL 45 Laboratory bottle (2 L)	DWK life sciences	218016357	Used for waste collection from the 5 L bioreactor.
DURAN Original GL 45 Laboratory bottle (5 L)	DWK life sciences	218017353	Used for waste collection from the 15 L bioreactor.
DURAN Original GL 45 Laboratory bottle (500 mL)	DWK life sciences	218014459	Used for supplementary bottle of 0.1 M NaOH.
EZ Top Container Closures for NALGENE-containers (500mL)	Saint-Gobain	EZ500 ML-38-2	Brands and catalogue numbers are only for example, similar products are available from various suppliers and as long as they have the same functionality, items could be substituted with other brands.
Fetal bovine serum (FBS) superior quality	Wisent	086-150	Used for cultivation of HEK293T cells.
FITC anti-human CD14 Antibody	BioLegend	301804	Used in flow cytometry for MSC identity assessment.
FITC anti-human CD34 Antibody	BioLegend	343504	Used in flow cytometry for MSC identity assessment.
FITC anti-human CD90 (Thy1) Antibody	BioLegend	328108	Used in flow cytometry for MSC identity assessment.
Flow cytometry	Beckman Coulter	CytoFLEX	Used for cell identity assessment.
Fluorescence Cell Analyzer	Alit life science	Countstar Rigel S2	Used for cell counting. Cell viability can be calculated by staining with AO/PI dyes.
GL 45 Multiport Connector Screw Cap with 2 ports	DWK life sciences	292632806	Brands and catalogue numbers are only for example, similar products are available from various suppliers and as long as they have the same functionality, items could be substituted with other brands.
Glucose Meter	Sinocare	6243578	Used for detecting glucose concentration in cell culture medium and supernatant.
Hank's Balanced Salt Solution (HBSS), with calcium and magnesium	Gibco	14025092	Used for preparation of digest solution.
Human Albumin 20% Behring (HSA)	CSL Behring	N/A	Used for preparation of wash buffer.
Inverted fluorescent microscope	OLYMBUS	CKX53SF	Used for brifgt field and fluorescent observation and imaging.
Nalgene Measuring Cylinder (500 mL)	Thermo Scientific	3662-0500PK	Used for calibrating the liquid handling volume speed of peristaltic pumps.
Newborn calf serum (NBS) superfine	MINHAI BIO	SC101.02	Used for cultivation of Vero cells.
OriCell human mesenchymal stem cell adipogenic differentiation and characterization kit	Cyagen	HUXUC-90031	Used for tri-lineage differentiation of hUCMSCs.
OriCell human mesenchymal stem cell chondrogenic differentiation and characterization kit	Cyagen	HUXUC-90041	Used for tri-lineage differentiation of hUCMSCs.
OriCell human mesenchymal stem cell osteogenic differentiation and characterization kit	Cyagen	HUXUC-90021	Used for tri-lineage differentiation of hUCMSCs.
PE anti-human CD105 Antibody	BioLegend	800504	Used in flow cytometry for MSC identity assessment.
PE anti-human CD19 Antibody	BioLegend	302208	Used in flow cytometry for MSC identity assessment.
PE anti-human CD73 (Ecto-5'-nucleotidase) Antibody	BioLegend	344004	Used in flow cytometry for MSC identity assessment.
PE anti-human HLA-DR Antibody	BioLegend	307605	Used in flow cytometry for MSC identity assessment.



Phosphate Buffered Saline (PBS)	Wisent	311-010-CL	Used in autoclaving of glass vessel and V01 microcarriers, and replacement of culture medium.
Sani-Tech Platinum Cured Sanitary Silicone Tubing (0.13 in x 0.25 in)	Saint-Gobain	ULTRA-C-125-2F	Used for solution transfering driven by peristaltic pumps.
Sterile Saline	Hopebiol	HBPP008-500	Used for preparation of wash buffer.
Trypzyme Recombinant Trypsin	BasalMedia	S342JV	This reagent is used for bead-to- bead transfer of HEK293T and Vero cells.
Tube Sealer	Yingqi Biotech	Tube Sealer I	This sealer is compatible with both C-Flex tubing and PVC tubing.
Tube Welder for PVC tubing	Chu Biotech	Tube Welder Micro I	Used for welding of PVC tubing.
Tube Welder for TPE tubing	Yingqi Biotech	Tube Welder I-V2	Used for welding of TPE tubing.
ViaStain AO / PI Viability Stains	Nexcelom	CS2-0106-25mL	Dual-Fluorescence Viability, using acridine orange (AO) and propidium iodide (PI), is the recommended method for accurate viability analysis of primary cells, such as PBMCs, and stem cells in samples containing debris.