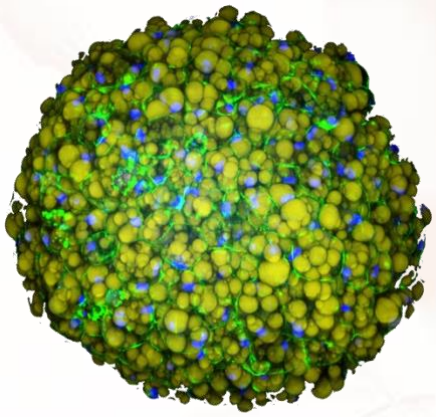
Bonds Biosystems

2D-to-3D BioSol™ AT transition kit (visceral)

Differentiation & maintenance



BBATVC2D3D-001

August 2022

Contents

Shipping conditions

Your orders are delivered via Federal Express courier. All US and Canada orders are shipped via Federal Express Priority service and are usually received the next day. International orders are usually received in 2-4 days. Please inquire if alternate couriers are needed.

Storage conditions

- Media: 21 days (from ship date) at 4°C and 6 months at -20°C.
- Live cells/spheroids/organoids: Must be processed immediately upon receipt and maintained at 37°C and 5% CO_2.

All Bonds BioSystems products are for research use only. They are not approved for human or veterinary use or in diagnostic or clinical procedures.

Ordering information and technical services

Bonds Biosystems Corporation

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USA

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E-mail:	info@bondsbio.com
Website:	http://www.bondsbio.com

Precautions

This product is for research use only. It is not intended for human, veterinary, or in vitro diagnostic use. Proper precautions and biological containment should be taken when handling cells of human origin, due to their potential biohazardous nature. Always wear gloves and work behind a protective screen when handling primary human cells. All media, supplements, and tissue culture ware used in this protocol should be sterile. These products are not for use in Clinical Diagnostic or Therapeutic Procedures.

By your acceptance of these products, you are acknowledging that these products will be:

1. Treated as potentially contaminated biological specimens even if accompanying serological reports are negative.

2. Handled by establishing or following appropriate safety control procedures to ensure the safety of using these products.

Pathogen testing

Samples from each donor are tested via PR to confirm non-reactivity for HIV-1, HIV-2, hepatitis B, and hepatitis C. However, no known test can assure that these cells are entirely pathogen free. Our products are tested and are free from mycoplasma contamination. Proper precautions and biological containment should be taken when handling cells of human origin due to their potential biohazardous nature. Human-based products should be manipulated at a BSL-2 (Biosafety Level 2) or higher. Always wear gloves and work behind a protective screen when handling primary human cells.

Limited warranty

This warranty limits our liability to replacement of this product. No other warranties of any kind, expressed or implied, including without limitation implied warranties of merchantability or fitness for a particular purpose, are provided by Bonds Biosystems Corporation. Bonds Biosystems Corporation shall have no liability for any direct, indirect, consequential, or incidental damages arising out of the use, the results of use, or the inability to use this product.

Bonds Biosystems Corporation warrants the performance of cells only if BioSol™ AT (visceral) Media Kit are used and the recommended protocols are followed.

Contact Bonds Biosystems Corporation within no more than 24 hours after receipt of products for all claims regarding shipment damage, incorrect ordering, or other delivery issues. Delivery claims received after 7 days of receipt of products are not subject to replacement or refund.

2D-to-3D transition kit introduction

Bonds Biosystems is the first company to successfully ship live adipose tissue models available in 2D culture and 3D spheroid/organoids to labs throughout North America in microplates, ready-to-assay. Designed to assist in **the transition from 2D to 3D cell culture**, this kit was developed to assist in this process. Our **3D BioSol™ Adipose Tissue (AT) subcutaneous and visceral models** are engineered and certified for testing different readouts simultaneously and synchronously and compared with the same cell source in a 2D differentiation protocol. We developed **3D BioSol™ AT subcutaneous and visceral models** using the most advanced 3D cell culture technologies to produce highly predictive, fully QC'd organotypic *in vitro* models ready to use for drug discovery, efficacy, and safety. Our organoids can be derived from patients ranging from Healthy (BMI under 25) to obese and Type 2 Diabetics (T2D) patients, at your convenience. **3D BioSol™ AT subcutaneous and visceral models** are the foundation of Bonds Biosystems 3D BioSol™ platform for drug discovery and safety, which we rely on in all our research collaborations and services.

Shipments of our ready-to-assay solutions include the appropriate 3D cell culture media optimized for differentiation and maintenance of specific adipose tissue subtypes (related to different depots) and applications.

Materials provided in this kit

- 96-wells plate with undifferentiated human adult primary SVF cells from visceral depot (omental), seeded in 2D conditions, at passage 6-8, 80-70% confluent (day -3), after 24-48 hours after seeding.
- 96-wells plate with undifferentiated human adult primary SVF cells from visceral depot (omental), seeded in **3D BioSol™ AT subcutaneous and visceral models**, at passage 6-8, after 24-48 hours after seeding.
- BioSol™ AT (visceral) Media Kit containing Media A, Media B and Media C.

Media description

General

The **BioSol™ AT (visceral) Media Kit (A, B and C)** provides a ready-to-assay cell adipogenesis differentiation workflow. Media A serves as a proliferation medium and should be used prior to the onset of the differentiation phase of adipogenesis. Fetal Bovine Serum (FBS) is contained in this medium. The role of Media B in activating the cellular programming of adipogenesis. In media C, physiological conditions are maintained for the final maturation stage of adipocytes (2D) or organoids (3D). FBS is not present in media B and C.

Application

The differentiation of human adult primary SVF into adipose tissue for both 2D and 3D cultures.

Media expiration dates

- If placed at 4°C upon arrival, these media are stable for 3 weeks.
- If stored at -20°C upon arrival, these media are stable for 6 months from the date printed in their labels.

Background

Adipose tissue stromal vascular fraction (SVF) primary cells are an easily accessible, heterogeneous cell system comprised of endothelial cells, immune cells, mainly macrophages, fibroblasts, mesenchymal stem cells (MSC), as well as smooth muscle cells, mural cells, blood cells, and a whole cadre of other stem cell phenotypes (1-3). While this mixed population more closely recapitulates the variety of cells seen in vivo, there is an overall lack of consensus regarding the specific proportions of these constituents to one another (4). Contributing to this is the fact that the SVF composition is dependent on a variety of factors, such as the adipose isolation site, processing methods, and the patient's own pathological status.

Protocols

Receiving BioSol™ products

Upon receiving microtissues (Spheroids and Organoids), follow the instructions below and perform a media exchange to replace the shipment medium with fresh tissue-specific maintenance medium (provided). Tissues are ready for compound dosing following a 12-24-hour acclimation period.

Unpacking your BioSol™ products

Upon receiving the plates (2D and 3D BioSol™ AT-visceral), please follow the instructions carefully to ensure your safety and the optimal performance of these cells.

1. Check the seal for each plate. Discard any plate where the vacuum seal has been compromised during shipment. Please be aware that these cells are of human origin. Please treat them as potentially infectious since we cannot test for all pathogens. Always wear gloves and use protective measures when handling human primary cells.

2. Place the BioSol™ package into a sterile environment. This is very important since breaking the vacuum seal may potentially introduce contamination into the plate. Use scissors to snip open the bag at any end. The vacuum seal should be released at this time. You may notice some bubbling of the medium in the plate at this time. This is normal and will not affect cell performance.

3. In a sterile environment, remove the plate from the bag, taking care to not disturb the cover top from the plate.

    a. **For 2D BioSol™ AT (visceral - undifferentiated):**

        i. Open the lid and remove the white liner using sterile forceps or a hemostat and discard. Carefully remove the clear adhesive seal by grabbing the edge with sterile forceps or hemostat and lifting the film slowly towards the other end. Discard adhesive film in appropriate biohazard waste container. Replace lid on plate.

ii. The excess medium added to each well for shipping (300µL/well) should be removed before incubation in a humidified atmosphere CO_2 incubator. Remove 150 µL/well from each well (Chart above).

b. For 3D BioSol™ AT (visceral - undifferentiated):

i. Open the lid and remove the white liner using sterile forceps or a hemostat and discard. Carefully remove the clear adhesive seal by grabbing the edge with sterile forceps or hemostat and lifting the film slowly towards the other end. Discard adhesive film in appropriate biohazard waste container. Replace lid on plate.

ii. The excess medium added to each well for shipping (250µL/well) should be removed before incubation in a humidified atmosphere CO_2 incubator. Remove 170 µL/well from each well (Chart above).

System	***Format***	***Total shipment volume per well***	***Removal volume per well***
2D culture	96 well plate	300µL	150µL
3D culture	96 well plate	250µL	170µL

4. Keep the plates at 37°C with 5% CO2 in a humidified incubator until ready for use. **Live cell orders are to be processed immediately upon arrival**.

a. For 2D BioSol™ AT (visceral - undifferentiated):

i. SFV primary cells are shipped in 96 well assay plate (COSTAR® 3603) at 70-80% confluence (day -3) within a 24 to 48-hour period post-seed.

ii. SFV primary cells are flat, phase-dark spindle-shaped cells. The cells have a similar appearance in culture to fibroblasts or smooth muscle cells. The majority of the preadipocytes and mesenchymal cells will differentiate into adipocytes using Media B (BB0002-0200) and Media C (BB0003-200) as described in this manual.

iii. Incubate 12-24 hour to acclimate.

iv. Run your assays

b. For 3D BioSol™ AT (visceral - undifferentiated):

i. SFV primary cells are shipped in 96 well assay plate (Akura ™ 96 Plate)* at 24 to 48 hours after spheroid assembling (≅ 12-24 hours).

ii. SFV primary cells were aggregated in spherical shape with phase-dark edges. The majority of the preadipocytes and mesenchymal cells will differentiate into adipocytes using Media B (BB0002-0200) and Media C (BB0003-200) as described in this manual.

iii. Incubate 12-24 hour to acclimate.

iv. Run your assays

*The following information will help you handle the Akura™96 Plate: *https://insphero.com/wp-content/uploads/2022/02/Akura-96-Spheroid-Microplate-Product-Manual_2022.pdf*

Adipose tissue differentiation

1. Visceral SFV primary cells are plated to be confluent in Media A (BB001-0100) and shipped the 24-48 hours (day -3) via overnight delivery. Differentiation should be initiated within 24-48 hours after receiving and detecting the cells are at 100% confluence (day -2), see Table 1. 3D BioSol™ AT protocol). Please contact Bonds Biosystems, Corp. to coordinate the shipping date with your schedule.

2. To start the process, aspirate the volume of Media A from all wells:

a. For 2D and 3D BioSol™ AT (visceral - undifferentiated):

a. Add the appropriate volume Media B (BB0002-0200) to the wells (see Table 2. Feeding Volumes). Incubate plate for 9 days at 37°C and 5% CO_2.

b. After 9 days, cells should be fed by removing some of the medium and replacing with fresh Media C (BB0003-200; See Table 2. Feeding Volumes). ***Caution***: Do not dry the wells. Add new medium gently. If using an automatic feeder, set the slowest flow rate possible.

c. Two (2) weeks after the initiation of differentiation, cells should appear rounded with large lipid droplets apparent in the cytoplasm. Cells are now considered mature adipocytes and are suitable for most assays.

d. From the second week, the removal and fed media should be reduced to 50% of the total volume every 3-4 days.

Table 1. 3D BioSol™ AT protocol

System	***Format***	***Media B (Differentiation)***		***Media C (Maintenance)***	
		Incubation time	Media exchange		Media exchange
2D culture	96 well plate	9 days	3 days	5-11 days	3-4 days
3D culture	96 well plate	9 days	3 days	11-21 days	3-4 days

Table 2. Feeding Volumes

System	***Format***	***Work vol. (µL)***	***Change Media A to Media B***		***Change Media B to Media C***		***Change Media C to Media C***	
			OUT	**IN**	**OUT**	**IN**	**OUT**	**IN**
2D culture	96 well plate	150/well	130/well	130/well	130/well	130/well	100/well	100/well
3D culture	96 well plate	80/well	70/well	70/well	60/well	60/well	40/well	40/well

The differentiation efficiency may varies depending on the donor. Please see the Certificate of Analysis that came with your order for information specific to the cells you have received.

References

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2- Afasf Riordan, N.H., Ichim, T.E., Min, W., Wang, H., Solano, F., Lara, F., Alfaro, M., Rodriguez, J.P., Harman, R.J., Patel, A.N., Murphy, M.P., Lee, R.R., and Minev, B. Nonexpanded adipose stromal vascular fraction cell therapy for multiple sclerosis. J Transl Med 7, 29, 2009.

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4- Cousin, B., Caspar-Bauguil, S., Planat-Benard, V., Laharrague, P., Penicaud, L., and Casteilla, L. Adipose tissue: a subtle and complex cell system. J Soc Biol 200, 51, 2006.

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