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TECHNICAL INFORMATION

Catalog Number: 152349, 152350, 152351, 152432, 152433, 152434, 152435, 152436, 152438, 152439
Rapid Cell

TISSUE CULTURE SYSTEMS

The RapidCell™ system for cell culturing involves a series of microcarrier beads specifically designed for culturing cells that require a support for growth. The RapidCell™ series allows for rapid cell culturing while minimizing costs.

RapidCell™ ADVANTAGES

– Promotes rapid cell growth
– Grows cells to higher densities
– Obtains more excreted product per unit area
– Allows harvesting of more virus per volume
– Allows harvesting of more cells per volume
– Less harsh treatment results in greater cell viability.

RapidCell™Series of Microcarrier Beads
RapidCell™ CCollagen-coated microcarrier beads
RapidCell™ GGlass microcarrier beads
RapidCell™ PPlastic microcarrier beads

Surface area: 325 cm^2/gram

RapidCell™ GENERAL GUIDELINES FOR GROWING CELLS

Preliminary Seeding

The first step in beginning the RapidCell™ system is to get cells such as fibroblast, epithelial cells, chondrocytes, etc growing on RapidCell™ . Follow your usual trypsinizing protocol then add enough RapidCell™ to almost cover the bottom of the newly seeded flask. After a couple of days, the cells will attach not only to the flask but to RapidCell™.

An alternative method is to add enough RapidCell™ to cover about 1/2 the bottom of a flask of cells which are about 50% confluent, and allow cells to grow near confluency. Some cells will adhere to RapidCell™, which can be used to seed another flask.

Next. gently rock or swirl the flask to spread out the RapidCell™ somewhat evenly.

For petri plates, it is best to use a sterile pasteur pipette and swish the media over the RapidCell™ a few times before drawing off enough RapidCell™ to seed another dish. This will break loose RapidCell™ covered with cells so that efficient seeding occurs.

Harvesting Cells

If individual cells are required, seed a flask with RapidCell™, covered with adherent cells. Grow the culture to near confluency then shake the flask moderately, tilt at an angle, and remove the RapidCell™. Wash the remaining cells on the flask with media of PBS and repeat the procedure. The cells adhering to the flask may then be removed by trypsinizing.

The cells adhering to RapidCell™ are difficult to remove due to their strong adherence to the substrate. Some limited success has been obtained by treating with trypsin.

RULE OF THUMB

Use enough RapidCell™ to cover 1/3-3/4 of the bottom of the tissue culture flask. As little as 1/8 of the flask may be covered with RapidCell™ if low density passage is desired. Low density passage is desirable for cultures not needed in the near future thus saving time, supplies and media.

SPLITTING A CULTURE

Once cells attach and grow on RapidCell™, a micro-environment is formed not only between adjacent cells on RapidCell™, but between adjacent RapidCell™ particles thus forming clumps. This process allows for seeding large flasks (T75) and 100 cm petri dishes with only a few RapidCell™, such as those obtained from a 24 well flat tissue plate. This process allows one to control the time it takes to grow cells to confluency by adjusting the amount of RapidCell™ used to seed a new flask. The lesser amount of RapidCell™ coated with cells, that is used to seed a new culture, the greater the time it takes to reach confluency; while the more RapidCell™ coated with cells that is used, the faster confluency can be reached. Cells attach not only to RapidCell™ but to the flask or dish surface as well as to nearby RapidCell™ matrix.

To split a culture quickly, close the top of the flask and gently break loose the RapidCell™ from the flask by agitation. Tilt the culture so as to concentrate the RapidCell™ onto any edge. Using sterile techniques, remove at least one drop of RapidCell™ and transfer it to a flask containing fresh media (as much as 1 ml RapidCell™ can be used if rapid seeding is required). Add enough fresh RapidCell™ to cover about 1/4 the bottom of the new culture flask. If a higher cell density is required, add additional RapidCell™.

SPINNER CULTURES

RapidCell™ can be used in Spinner cultures but the process is more empirical and varies depending upon cell types and culture conditions. There are two approaches to Spinner cultures. This first is to use RapidCell™ as a seeding agent for new cultures. If this is the purpose, use a small amount of RapidCell™ and rotate the flasks as slow as possible until RapidCell™ matrix adheres to the flask. The second use is for high density growth. Since cell type, media, and culture conditions vary widely no single process will suffice . If high density growth is required, use a large amount of RapidCell™ (enough to cover the length of the flask at 1/8 into to 1/2 inch deep in the center while lying the flask on its side). Then spin the flask very slowly (such as 1 rpm or less). This process may require frequent media change, as the cell density can be very high.

RapidCell™ C, RapidCell™ G, and RapidCell™ P -- PROTOCOL FOR GROWING CELLS

The following protocol is applicable to RapidCell™ C, G and P microcarrier beads. This protocol is based on preparing 200 ml suspension cultures in spinner flasks. Bead loadings of 5-40 grams per liter have been demonstrated successfully.

– Clean, siliconize and autoclave all glassware and pipettes. Any commercially available agent (e.g. Prosil-28) is acceptable
– Suspend the weighed RapidCell™ C, G or P beads in distilled water and autoclave at 121°C for 15 minutes. For this 200 ml protocol, we suggest 5 gm of beads (equivalent to loading of 25 gm/liter), but bead loading of 5-40 gm/liter have been used successfully.
– Discard the water and immerse the RapidCell™ beads in a small amount of medium. The type of medium used is not critical. Whatever nutrient is used for the cells in monolayer will be satisfactory for the RapidCell™ bead suspension culture.
– Allow to soak for 30 minutes (45 minutes for RapidCell™ P) in the medium and then discard this medium. Add fresh warm medium, bead suspension, the cells and enough warm medium to make 100 ml.
– Stir the incubated spinner flask at 21-23 rpm for a minimum of 2 hours and then bring 200 ml with fresh, warm medium.

PROTOCOL FOR HARVESTING CELLS

RapidCell™ C, G, and P microcarriers have a surface from which cells can be efficiently and gently removed. Yields of viable cells are often greater than 95%. The following protocol has been successfully used to harvest a variety of cells from suspension culture flasks (100-1000ml)

– Allow the microcarriers to settle and decant the medium from the suspension culture flasks. Gently rinse the microcarriers in a small volume of phosphate-buffered saline (calcium and magnesium free) and then resuspend the cells in a solution of trypsin. A 0.25% solution of trypsin (MP Trypsin 1:250) in PBS is recommended. Additionally, EDTA is often added to facilitate the removal of divalent cations.
– The cells and microcarriers are incubated in the trypsin solution for a brief period of time (1-2 minutes may be sufficient for fibroblastic cells although 5-10 minutes may be necessary for epithelial cells). After the cells and microcarriers have been exposed to the trypsin solution, they should be gently triturated to release the cells from the microcarrier surface.
– After the cells are released from the microcarriers, the trypsin solution should be diluted with serum-containing medium to inhibit further trypsin activity. The cells and microcarriers are then centrifuged and resuspended in culture medium.
– The cells may now be separated from the microcarriers by filtration through a 75 mm mesh nylon, teflon or stainless steel screen.

CLEANING PROTOCOL FOR REUSING MICROCARRIER BEADS*

– Place microcarrier in a 250 ml siliconized glass Erlenmeyer flask
– Add PBS to 150 ml.
– Allow microcarriers to settle and remove (via suction) the PBS. Take care not to disturb the microcarriers
– Add glass-distilled water to 90 ml.
– Add 10 ml of 1.0M NaOH.
– Allow microcarriers to soak for 30 minutes at room temperature. Shake the flask vigorously every 10 minutes.
– Filter out the microcarriers using a 70 mm sintered stainless wire mash screen.
– Wash the microcarriers while still on the screen by passing 1.5-2.0 liters of glass-distilled water through the filter apparatus.
– Resuspend the microcarriers in 100ml PBS and place into a siliconized 125ml Erlenmeyer flask and autoclave.
– Autoclaving at 121ºC for 15 minutes is sufficient.

* With this protocol, some users report that they have been able to reuse microcarriers at least 10 times.

SUCCESSFUL CELL CULTURE EXPERIMENTS

Cell culture scientists and engineers all over the world have been utilizing on or more types of carrier beads in their laboratories. Following is a partial list of cell lines or primary cells that have been successfully grown on microcarrier beads.

CODE	NAME	TYPE OF BEAD
VERO	Monkey kidney	G,C
CEF	Chicken embryo fibroblast	G
BHK	Hamster kidney	G
ST	Swine testes	G,C
BT	Bovine turbinate	C
P4RSF	Rabbit skin fibroblast	G,C
MRC-5	Human diploid fibroblast	G,C
KB	Human epithelial carcinoma	C
HFF	Human foreskin fibroblast	G,C,P
UMSCC-2	Human squamous carcinoma	G,C
MDBK	Madin-Darby bovine kidney	G,C,P
CHO	Chinese hamster ovary	

*(G) glass coated beads (C) collagen coated and/or (P) plastic beads

REFERENCE REGARDING OUR RapidCell™ MICROCARRIER BEADS

– Growth of Three Established Cell lines on Glass Microcarriers, James Varani, Michael Dame, Ted F. Beals and John A. Wass, **Biotechnology and Bioengineering,** Vol. 25, no. 5 , pp. 1359-1372 (1983).
– Substrate-dependent differences in growth and biological properties of fibroblasts an epithelial cells grown in microcarrier culture. Jmaes Verani, Michael Dame, John Rediske, Ted F. Beals and William J. Hillegas. **Journal of Biological Standardization**, Vol.13, pp.67-76 (1985).
– Proteolytic Enzymes and Arachidonic Acid metabolits Produced by MRC-5 Cells in Various Microcarrier Substrates. James

Varani, Jeffrey D. Hasday, Robert Sitrin, Pamela G Brubaker and Willian J. Hillegas. **In Vitro Cellular and Developmental Biology**, Vol.22 , No.10 pp.575-582 (1986)
– Cell Growth on Microcarriers : Comparison of Growth and Recovery from Various Substrates. James Varani, Mathew J. Bendelow and William J. Hillegas, **Journal of Biological Standardization,** Vol. 16, pp. 333-338 (1988)
– The Effect of Substrate on the Production of Infectious Virus by Cells in Culture. James Varani, Matthew J. Bendelow and William J. Hillegas, **Journal of Biological Standardization** Vol. 16, pp
– Substrate-Dependent Differences in Production of Extracellular matrix Molecules by Squamous Carcinoma Cells and Diploid Fibroblasts. James Varani, Suzanne E. G. Fligiel , Dennis R. Inman, David L. Helmreich, Matthew J. Bendelow and William J. Hillegas, **Biotechnology and Bioengineering**, Vol. 33, pp. 1235-1241 (1989).

AVAILABILITY

Catalog Number	Description	Size
152432	RapidCell™ C, 90-150 um, 1.02 gm/cm^3	5 gm 25 gm 100 gm
152433	RapidCell™ C, 150-210 um, 1.02 gm/cm^3	5 gm 25 gm 100 gm
152434	RapidCell™ C, 90-150 um, 1.03 gm/cm^3	5 gm 25 gm 100 gm
152349	RapidCell™ C, 150-210 um, 1.03 gm/cm^3	5 gm 25 gm 100 gm
152435	RapidCell™ G, 90-210 um, 1.02 gm/cm^3	5 gm 25 gm 100 gm
152436	RapidCell™ G, 150-210 um, 1.02 gm/cm^3	5 gm 25 gm 100 gm
152350	RapidCell™ G, 150-210 um, 1.03 gm/cm^3	5 gm 25 gm 100 gm
152438	RapidCell™ P, 90-150 um, 1.02 gm/cm^3	5 gm 25 gm 100 gm
152439	RapidCell™ P, 150-210 um, 1.02 gm/cm^3	5 gm 25 gm 100 gm
152351	RapidCell™ P, 150-210 um, 1.03 gm/cm^3	5 gm 25 gm 100 gm