

PVA Microcarriers AS Protocol

Features of PVA Microcarriers AS

- The surface can be coated with a cell-adhesive protein or coating material of choice, making PVA Microcarriers AS suitable for the attachment and proliferation of various cell types.
- Because PVA Microcarriers AS is made of an elastic PVA hydrogel, it is resistant to breakage under mechanical stresses, such as agitation, thereby reducing the risk of particulate contamination caused by fragmented debris.
- Its high transparency enables easy microscopic observation of attached cells.
- Animal-free cell culture is possible by selecting an appropriate coating material.

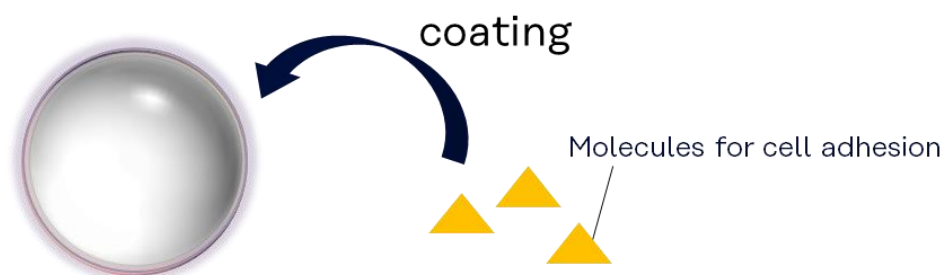


Table 1. Features of PVA Microcarriers AS

Particle size	Approx. 160 μm *2 (D50)
Surface area	Approx. 1900 cm^2/g dry weight *3
Surface	Activated
Swelling ratio	Approx. 5 g wet weight/g dry weight *4

The values shown in the table are representative values.
Lot-specific particle size and surface area information is provided with the product.

*1. Particle size is values measured for PVA Microcarriers AS after swelling in PBS.

- *2. Surface area is the value obtained when 1 g of dry PVA Microcarriers AS is swollen in PBS.
- *3. The swelling ratio is the increase in weight when dry PVA Microcarriers AS is swollen in PBS.

Surface Coating Methods

The surface of PVA Microcarriers AS can be coated with various extracellular matrix proteins. This section describes two surface-coating methods. The suitability of Procedure A and Procedure B may vary depending on the coating solution and cell type. If satisfactory cell attachment is not achieved with one procedure, please try the other. Coating has been evaluated using fluorescent proteins, which may facilitate assessment of the coating conditions.

Procedure A:

This procedure is suitable when coating the microcarriers with only the desired cell-adhesion molecule or when the coating amount needs to be determined. The coating amount can be estimated from the concentration of the coating solution before and after coating.

Procedure B:

In this procedure, the coating material is added directly to the culture medium and culture is performed without an additional washing step. This method is convenient for culture evaluation.

Procedure A. Precoating with a PBS solution of a cell-adhesion molecule

1. Measure the tare weight of a 50 mL tube using an analytical balance.
2. Using a sterile spatula, transfer the required amount of PVA Microcarriers AS to the 50 mL tube.

Note: PVA Microcarriers AS may scatter because of airflow or static electricity. Handle the product carefully and use an antistatic tube or static eliminator if necessary.

3. Add a coating solution at about 30 times the weight of dry PVA Microcarriers AS and suspend the microcarriers directly in the solution.

Example: Add 3 mL of a cell-adhesion molecule solution to 100 mg of dry PVA Microcarriers AS.

Note: Adding the coating solution after swelling may reduce coating efficiency. PVA Microcarriers AS may float on the liquid surface when the coating solution is added. If this occurs, gently shake, pipette, or mix with a vortex mixer to disperse the microcarriers before proceeding to the next step. Use a volume of coating solution sufficient to fully immerse the particles and adjust the volume as needed for your application.

4. Mix thoroughly for at least 1 hour using a tube rotator or shaker to coat the surface.

Note: When coating the surface with a protein, avoid foaming the protein solution.

5. Stop mixing, allow PVA Microcarriers AS to settle by centrifugation or static settling, and remove the supernatant.
6. Add fresh PBS and wash PVA Microcarriers AS using a vortex mixer or equivalent method.
7. Repeat Steps 4 and 5 three times.
8. After surface coating, PVA Microcarriers AS can be stored in PBS at 4°C for approximately 1 week.

Procedure B. Adding a cell-adhesion molecule directly to the culture medium

1. Add the culture medium and an appropriate amount of the cell-adhesion molecule to a new 50 mL tube and mix.

Example: To coat PVA Microcarriers AS with a surface area of 208 cm² using iMatrix-511 at 0.1 µg/cm², add 41.6 µL of iMatrix-511 stock solution (0.5 mg/mL) to 30 mL of culture medium.

2. Add the medium containing the cell-adhesion molecule and the measured PVA Microcarriers AS to the bioreactor vessel, and pipette thoroughly.

Note: PVA Microcarriers AS may float on the liquid surface. Pipette thoroughly to break up large clumps and disperse the microcarriers in the medium.

3. Place the bioreactor on the stirring device and mix the medium and PVA Microcarriers AS for at least 1 hour (up to overnight). The cell-adhesion molecule coats the PVA Microcarriers AS surface, after which cells can be seeded directly into the bioreactor for culture.

The coating can be observed by fluorescence microscopy using a fluorescent protein. Alternatively, the coated protein can be fluorescently labeled after coating using a standard fluorescence-staining method.

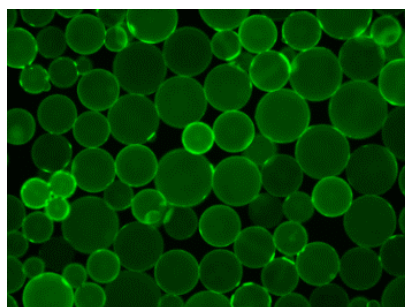


Figure 1. Fluorescence image of iMatrix-511 coating

Reagents and Equipment Required for Cell Culture

PVA Microcarriers AS can be used in various shaking and stirred culture systems.

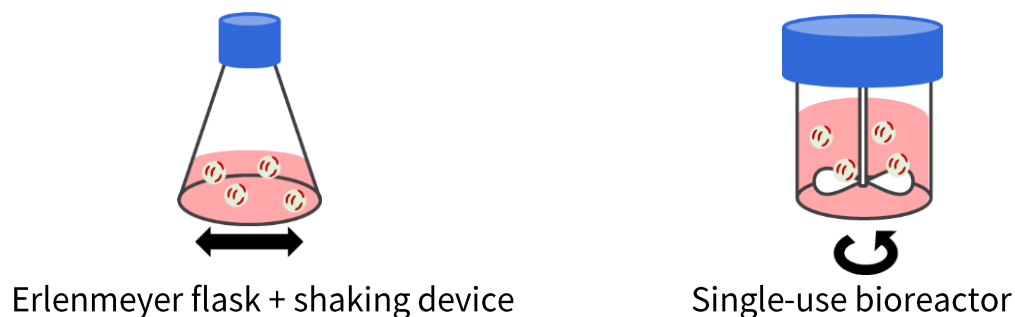


Figure 2. Schematic illustration of cell culture using PVA Microcarriers AS

Table 2. Required Reagents and Equipment

Reagents and Equipment	Example
Vessels and equipment for shaking or stirred culture	30 mL single-use bioreactor + 6-channel magnetic stirrer
	Erlenmeyer flask with vented cap + shaker
Cells	Mesenchymal stromal cells, fibroblasts, and other adherent cell types
Culture medium	Select according to the cell type and culture conditions
Cell dissociation reagent	0.025% ~ 0.25% Trypsin-EDTA
	Accutase®
Cell strainer	70 µm cell strainer
	100 µm cell strainer

PVA Microcarriers AS swells within a few of seconds when added to PBS or culture medium.

Separate culture examples are available. Please refer to them when conducting your evaluation.

Contact for Product Information

Life Innovation Business Promotion Department,
Functional Materials Company
Kuraray Co., Ltd.

Tokiwabashi Tower, 2-6-4, Otemachi, Chiyoda-ku, Tokyo
100-0004, Japan

E-mail Contact.LIPG@kuraray.com