COSMO BIO CO., LTD.
Inspiration for Life Science

Sample

KOKEN
AteloCell®

Collagen microspheres

Cat No.:	KOU-MIC-00
Lot No.:	*
ORIGIN:	Bovine dermis
STORAGE:	4°C (Do not freeze)
REFERENCES:	Refer to the AteloCell® website http://www.cosmobio.com

	Specification	Results
STERILITY TEST: (Medium: TGC-I and SCD)	Negative	Pass
CELL CULTURE TEST: (Cell: Human Fibroblast)	Normal	Pass

FOR RESERCH USE ONLY, NOT FOR HUMAN BODY.

Manufactured by KOKEN Co., Ltd.

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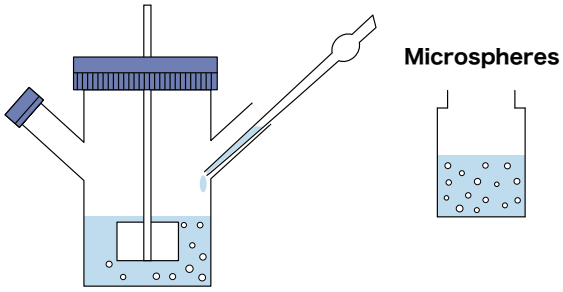
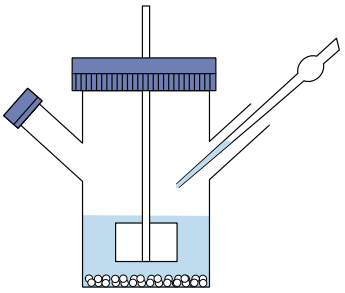
I. Preparation

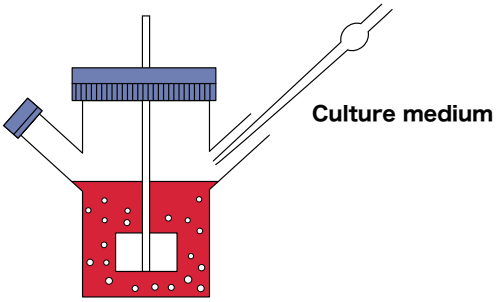
(1) Transfer 15 mL of microspheres into a microcarrier spinner flask.



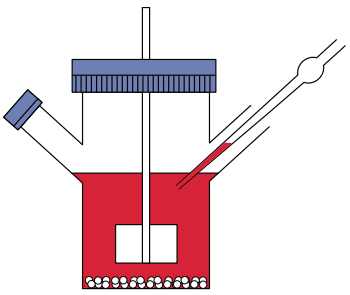
(2) Remove the supernatant after the microspheres have settled at the bottom of the flask.



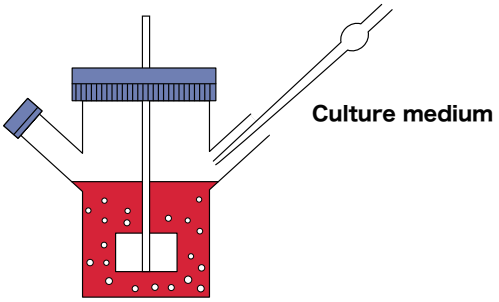
(3) Add 25-50 mL of cell culture medium.



(4) After the microspheres have settled, remove the supernatant as in (2). Repeat (2) - (4) 3 to 5 times. Increase the number of cycles when a larger volume of microspheres is used.

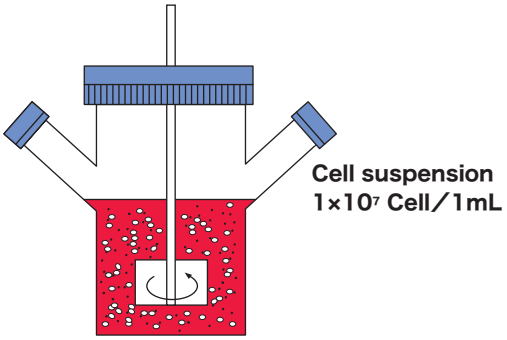


(5) Add 25-50 mL of cell culture medium.

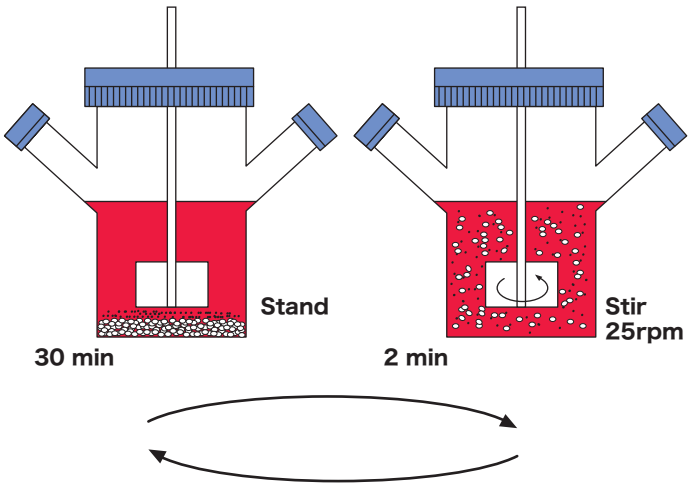


II. Seeding cells on collagen microspheres

(1) Add cell suspension (1x10^7 cells/1 mL) and stir immediately at 37℃.



(2) Allow to stand for 30 minutes and stir at 25 rpm for 2 minutes, then allow to stand for 30 minutes. Repeat this procedure for 6 hours, and then stir continuously at 25 rpm thereafter*1.

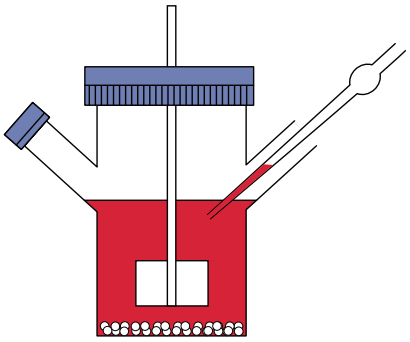


*1 As the cells proliferate in the flask, increase the rotation speed gradually. The rotation speed should be chosen carefully because too fast rotation can cause cell damage, whereas too slow rotation leads to aggregation of the microspheres due to the formation of cellular bridges between the microspheres, thus inhibiting cell proliferation.

III. Medium change

(1) Stop stirring and allow the microspheres to settle. Remove the supernatant.

(2) Add 25-50 mL of fresh medium*2.



*2 Change the medium 2-3 times a week. The medium should be changed more often as the cells proliferate in the flask. Also monitor the pH by the color of the medium.