

Preparation of media for cultivation of JCRB1564 TMNK-1

A. Basal medium

MCDB131:DMEM (low glucose) = 1:1 mixture

B. Preparation of complete medium

Add the following supplements to 500 mL of basal medium

EGM-2 SingleQuots (Clonetics, Cat.No.CC-4176)

FBS	10mL
Hydrocortisone	0.2mL
rhFGF-b	2.0mL
VEGF	0.5mL
R3-IGF-1	0.5mL
rhEGF	0.5mL
Heparin	0.5mL

(Not use: Ascorbic acid, GA-1000 in EGM-2 Single Quots)

0.2M L-Ascorbic acid phosphate magnesium salt n-hydrate*¹ 0.5mL
(Fujifilm Wako Cat.No. 013-12061)

When you thaw the frozen cells and start the culture, and when you prepare the freezing medium containing 10% DMSO).

⇒ Use complete medium (B).

For routine maintenance and propagation of cells.

⇒ Use 1:1 mixture of basal medium (A) and complete medium (B)

*1) Ascorbic acid is readily oxidized and is lost 1 day after culture at 37°C. So, the ascorbic acid is substituted with stable one, L-Ascorbic acid phosphate Mg salt n-hydrate. This is transformed to ascorbic acid by the esterase on surface of cells.

L-Ascorbic acid phosphate Mg salt n-hydrate is dissolved to warm water, filter sterilized, dispense to small aliquots, and store frozen.