Preparation of media for cultivation of JCRB1564 TMNK-1

A. Preparation of Mochitate modified MCDB131 (basal medium)

Water	984mL
Mochitate modified MCDB131(Powder)*1	10.35g
HEPES	2.38g
NaHCO3	3.18g
Penicillin G*2	100,000units
Streptomycin*2	100mg

Adjust pH to 7.2 by 1M NaOH, add water to total volume 1000 mL, and then filter sterilize.

B. Preaparation of complete medium

Add the following supplements to 500 mL of Mochitate modified MCDB131 (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*3 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) Special order.
*2) Optional.
*3) Ascorbic acid is readily oxdized and is lost 1 day after culture at 37C. 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 alliquots, and store frozen.