

feeder-free conditions according to the method described in Scientific Reports, 4, 3594 (2014). As the feeder-free medium, StemFit medium was used and, as the feeder-free scaffold, Laminin 511-E8 was used. Specific maintenance culturing
5 operation was performed in the same manner as in Example 1.
[0152]

The thus-prepared subconfluent human ES cells were washed with PBS, subjected to an enzyme treatment using Accumax, a serum-free medium for differentiation induction was added, and
10 the cells were scraped from the surface of the culture dish by using a cell scraper and dispersed into single cells by pipetting. Thereafter, the aforementioned human ES cells dispersed into single cells were suspended in 100 μ l of a serum-free medium at 1.2×10^4 cells per well of a non-cell-
15 adhesive 96-well culture plate (PrimeSurface 96V-bottom plate, manufactured by SUMITOMO BAKELITE), and cultured in suspension under the conditions of 37°C, 5% CO₂. As the serum-free medium (gfCDM+KSR) therefor, a serum-free medium which is a 1:1 mixture of F-12+Glutamax medium and IMDM+Glutamax medium
20 supplemented with 5% Knockout Serum Replacement, 450 μ M 1-mono thioglycerol, 1x Chemically defined lipid concentrate, 50 u/ml penicillin-50 μ g/ml streptomycin was used. At the time of the start of suspension culturing (day 0 from the start of suspension culturing, start of step 1), Y27632 (final
25 concentration 20 μ M) and SB-431542 (1 μ M) were added to the aforementioned serum-free medium. As the Wnt signal transduction pathway inhibiting substance, IWP-2 (manufactured by Tocris Bioscience, 2 or 5 μ M), C-59 (manufactured by Cayman Chemicals, 2 μ M), IWP-L6 (manufactured by AdooQ bioscience, 1
30 or 10 μ M), LGK974 (manufactured by Cayman Chemicals, 1 or 5 μ M), KY 02111 (manufactured by Cayman Chemicals, 1 or 5 μ M), or XAV939 (manufactured by Cayman Chemicals, 1 μ M) was added at the start of the suspension culturing, and the condition with the addition of DMSO alone as a control of no addition of a Wnt
35 signal transduction pathway inhibiting substance was performed.