Culture of microglial clone cells

$2x10^5$ cells in 6-cm plastic dish (Falcon 3002)
with Mi medium* containing 0.5-2 ng/ml** recombinant mouse GM-CSF (Genzyme)
culture for 7-10 days with changing medium every 3-4 days
reach aprox. 80% confluent
harvest
discard medium by aspiration
add 1 ml of pre-chilled Mi medium
scrape by rubber policeman
suspend cells by repeated gentle pipetting
collect cells in Mi-medium
into pre-soaked and pre-chilled plastic tube***
count cell number
(about 1-2x106 cells in total were expected to yield)

seed cells at $2x10^5$ cells in 6-cm plastic dish with Mi medium

incubate for 2-3 hr to make cells atached substriatum
then add GM-CSF. (Sometimes cells aggregate when GM-CSF is treated in floating condition)

@When you need ramified form of cells, you should culture cells with GM-CSF at least 2 days for conditioning, then replace medium without GM-CSF.

* Mi medium
Eagle's MEM (Nissui MEM or GIBCO MEM(low glucose)) or Sigma M4655 (liquid)
10% FCS or CS#
0.2% glucose
5ug/ml bovine insulin (Sigma I5500)

** Concentration of GM-CSF is depend on the culture condition i.e. lot of FCS, cell viability, and lot of GM-CSF. The concentration should be determined so as to obtain the growth rate about 10 times/7-10 days.##

*** Plastic wares (tube, tip, pipet etc.) should be prechilled and pre-soaked with chilled Mi medium. Glass wares shoud be avoid to use because of a lot of cell loss during preparation.

FCS: endotoxin-free FCS should be used. GIBCO FCSs are not so bad as far as I tried. I use a special grade of CS (Nakashibetsu Serum lot BO09 and BQ10 perchased from Mitsubisi Chemical, Japan).

Growth rate depends cell viability during harvest preparation. Rough scraping damages cells and lag time from seeding to starting to proliferate will prolong. You should get skilled to obtain above growth rate.