

## INTISARI

### **MORFOLOGI DAN PERSENTASE NEURON DOPAMINERGIK DARI KULTUR VENTRAL MESENSEFALON FETUS TIKUS PUTIH YANG DIPLATting DALAM *MINIMUM ESSENTIAL MEDIA*+10% *HORSE SERUM* MENGGUNAKAN PEWARNAAN IMUNOSITOKIMIA DENGAN PENANDA TIROSIN HIDROKSILASE KONSENTRASI 1:750**

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Penyakit Parkinson disebabkan oleh rusaknya neuron dopaminergik di substansia nigra pars kompakta. *Exogenous cell replacement* fetus merupakan salah satu terapi penyakit Parkinson. Oleh karena jumlah donor jaringan fetus yang terbatas, perlu dilakukan penelitian untuk menemukan metode kultur neuron dopaminergik dari ventral mesensefalon yang lebih optimal dan sederhana. Penelitian ini bertujuan untuk mengetahui persentase dan morfologi neuron dopaminergik dari kultur jaringan ventral mesensefalon fetus tikus putih umur 14 hari yang *diplatting* pada *minimum essential media*+10% *horse serum* menggunakan pewarnaan imunositokimia dengan penanda enzim tirosin hidroksilase konsentrasi 1:750.

Ventral mesensefalon fetus tikus (*Rattus novregicus albinus*) strain wistar umur 14 hari dipreparir, jaringan dimasukkan kedalam tabung konikal berisi media *minimum essential media*+10% *horse serum*+10% *fetal bovine serum*, kemudian dilakukan disosiasi secara mekanik, disentrifugasi selama lima menit, 200 x g, suhu 4°C dilakukan dua kali. Kultur dipindah ke *flask* yang berisi media yang sama, kemudian diinkubasi pada suhu 37°C dan CO<sub>2</sub> 5% selama 60 menit, selanjutnya sebanyak 2 ml media ditambahkan. Kultur ditanam selama tiga hari. Pada hari ke-empat, kultur *diplatting* ke dalam plat 96 sumuran yang berisi media MEM+10% *horse serum*. Pada hari ke-tujuh, kultur difiksasi menggunakan 4% paraformaldehida selama 30 menit untuk selanjutnya divisualisasikan dengan pewarnaan imunositokimia menggunakan *anti-tyrosine hidroksilase rabbit serum* (1:750, Chemicon Int., USA, Cat. No: AB152) sebagai antibodi primer. Pengamatan untuk mengetahui persentase neuron dopaminergik dilakukan pada 10 area yang dipilih secara acak dari setiap sumuran menggunakan mikroskop *inverted* perbesaran 10x10, sedangkan morfologi neuron diamati dengan perbesaran 10x20.

Jenis neuron dopaminergik berdasarkan morfologi yang tumbuh di dalam kultur ventral mesensefalon fetus tikus putih yang *diplatting* dalam media MEM+10% *horse serum* dan diwarnai menggunakan imunositokimia penanda tirosin hidroksilase konsentrasi 1:750 adalah neuron unipolar, bipolar, dan multipolar. Persentase neuron dopaminergik yang tumbuh adalah 21,3 ± 23,8% per bidang pandang .

Kata kunci: neuron dopaminergik, ventral mesensefalon, penyakit Parkinson, kultur sel, imunositokimia, tirosin hidroksilase

## ABSTRACT

### **MORPHOLOGY AND PERCENTAGE OF MESENCEPHALIC DOPAMINERGIC NEURON CULTURE OF RAT (*Rattus novregicus albinus*) FETUS PLATTED IN MINIMUM ESSENTIAL MEDIA+10% HORSE SERUM MEDIA USING IMMUNOCYTOCHEMISTRY WITH 1:750 TYROSINE HYDROXYLASE MARKER**

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Parkinson's disease is caused by the damage of dopaminergic neurons in the substantia nigra pars compacta that located in ventral mesencephalon. One of the Parkinson's disease treatment is using fetal exogenous cell replacement. Because the amount of donor tissue, is limited, it is necessary to conduct a research to find a more optimal and simple culture method of dopaminergic neurons to the ventral mesencephalon. The aim of this research is to know percentage and morphology of dopaminergic neuron in ventral mesencephalon culture using MEM+10% horse serum media and 1:750 tyrosine hydroxylase as marker of immunocytochemistry staining.

The mesencephalon of wistar rat (*Rattus novregicus albinus*) fetus aged 14 days were prepared and put the tissue into the conical tube consist of Minimum Essential Medium (MEM) + 10% horse serum + 10% fetal bovine serum media, then dissociated mechanically, centrifuged for five minutes, 200 x g, at 4°C. This method was repeated one more time. The culture medium was transferred to a flask containing the same medium, and then incubated at 37°C and 5% CO<sub>2</sub> for 60 minutes, and the cultures was incubated again for three days. The culture was plated into a 96-wells tissue culture plate for three days. Neuron dopaminergics were visualized by immunocytochemistry staining method using primary antibody anti-tyrosine hydroxylase (1:750, Chemicon Int., Temecula, USA, Cat. No: AB152). The percentage of dopaminergic neurons performed on 10 areas were selected randomly per well using inverted microscope with 10x10 magnification and morphology were observed using 10x20 magnification.

The types of neurons based on morphologic that grew at mesenchepalic dopaminergic neuron culture of rat fetus plated in MEM+10% horse serum media using immunocytochemistry with 1:750 tyrosine hidroxylyase marker were unipolar neurons, bipolar neurons and multipolar neurons. The percentage of dopaminergic neurons that grown using MEM+10% horse serum media was 21,3 ± 23,8% per area.

**Keywords:** dopaminergic neuron culture, ventral mesencephalon, substansia nigra pars compacta, Parkinson's disease, immunocytochemistry