

## DAFTAR PUSTAKA

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# LAMPIRAN

## LAMPIRAN 1. Persetujuan Etik


 KEMENTERIAN PENDIDIKAN DAN KEBUDAYAAN  
 UNIVERSITAS HASANUDDIN FAKULTAS KEDOKTERAN  
 KOMITE ETIK PENELITIAN KESEHATAN  
 RSPTN UNIVERSITAS HASANUDDIN  
 RSUP Dr. WAHIDIN SUDIROHUSODO MAKASSAR  
 Sekretariat : Lantai 2 Gedung Laboratorium Terpadu  
 JL PERINTIS KEMERDEKAAN KAMPUS TAMALANREA KM.10 MAKASSAR 90245.  
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**REKOMENDASI PERSETUJUAN ETIK**  
 Nomor : 66/UN4.6.4.5.31/ PP36/ 2020

Tanggal: 22 Januari 2020

Dengan ini Menyatakan bahwa Protokol dan Dokumen yang Berhubungan Dengan Protokol berikut ini telah mendapatkan Persetujuan Etik :

No Protokol	UH20010037	No Sponsor	
Peneliti Utama	<b>dr. Adelaide Adiwana</b>	Protokol	
Judul Peneliti	Efek Perendaman Kaki Tikus Galur Wistar Jantan Yang Dibuat Peripheral Artery Disease (PAD) Dalam Rendaman Air dan CO2		
No Versi Protokol	<b>1</b>	Tanggal Versi	<b>14 Januari 2020</b>
No Versi PSP		Tanggal Versi	
Tempat Penelitian	<b>Laboratorium Hewan FK Universitas Hasanuddin Makassar</b>		
Jenis Review	<input type="checkbox"/> Exempted <input checked="" type="checkbox"/> Expedited <input type="checkbox"/> Fullboard Tanggal	Masa Berlaku	Frekuensi review lanjutan
		22 Januari 2020 sampai 22 Januari 2021	
Ketua Komisi Etik Penelitian Kesehatan FKUH	Nama <b>Prof.Dr.dr. Suryani As'ad, M.Sc.,Sp.GK (K)</b>	Tanda tangan	
Sekretaris Komisi Etik Penelitian Kesehatan FKUH	Nama <b>dr. Agussalim Bukhari, M.Med.,Ph.D.,Sp.GK (K)</b>	Tanda tangan	

Kewajiban Peneliti Utama:

- Menyerahkan Amandemen Protokol untuk persetujuan sebelum di implementasikan
- Menyerahkan Laporan SAE ke Komisi Etik dalam 24 Jam dan dilengkapi dalam 7 hari dan Lapo SUSAR dalam 72 Jam setelah Peneliti Utama menerima laporan
- Menyerahkan Laporan Kemajuan (progress report) setiap 6 bulan untuk penelitian resiko tinggi dan setiap setahun untuk penelitian resiko rendah
- Menyerahkan laporan akhir setelah Penelitian berakhir
- Melaporkan penyimpangan dari prokol yang disetujui (protocol deviation / violation)
- Mematuhi semua peraturan yang ditentukan

## LAMPIRAN 2. Pemeriksaan Histopatologi / PA

- **Perhitungan Jumlah Vaskular**

Untuk menghitung jumlah vaskular, pembuatan preparat PA mengikuti langkah-langkah berikut ini:

1. Sediaan preparat dideparafinisasi dengan *xylene* sebanyak 2 kali, masing-masing selama 5 menit dan direhidrasi masing-masing selama 5 menit berturut-turut dengan alkohol 96%, alkohol 80% dan alkohol 70%.
2. Kemudian dicuci dengan air mengalir selama 5 menit.
3. Slide dimasukkan ke dalam larutan TRS, lalu dipanaskan dengan *microwave* selama 10 menit.
4. Setelah didinginkan, kemudian dicuci dengan PBS 2 kali, masing-masing selama 5 menit.
5. Pinggir jaringan ditandai, selanjutnya dilakukan *perokside block* selama 15 menit.
6. Preparat dicuci kembali dengan PBS 2 kali, masing-masing selama 5 menit, kemudian diberi *protein block* selama 5 menit, lalu dicuci lagi dengan PBS 2 kali, masing-masing selama 5 menit.
7. Antibodi yang dipakai adalah antibodi CD31 dengan pengenceran 1/200 yang diinkubasi pada suhu -20°C
8. Sediaan dicuci dengan PBS 2 kali, masing-masing selama 5 menit. Diberikan *Ultratek anti-polyvalent* (ScyTek) selama 10 menit. Sediaan dicuci kembali dengan PBS 2 kali, masing-masing selama 5 menit.
9. Kemudian diberi Ultratek HRP (ScyTek) selama 10 menit, lalu dicuci kembali dengan PBS 2 kali, masing-masing selama 5 menit.

10. Selanjutnya sediaan diinkubasi dengan *chromogen diaminobenzidine* (DAB) dan dicuci dengan air mengalir selama 5 menit, lalu direndam dalam larutan *hematoxylin* selama 5 menit.
11. Sediaan dicuci kembali dengan air mengalir. Setelah itu dilakukan dehidrasi dengan alkohol secara bertingkat (alkohol 70%, alkohol 80%, alkohol 96%) masing-masing selama 5 menit lalu dilakukan *clearing* ( *xylol* I, *xylol* II) masing-masing selama 5 menit.
12. Slide dikeringkan, kemudian diberi entelan, lalu ditutup dengan *deck glass*.
13. Dilakukan penghitungan jumlah vaskular di bawah mikroskop dengan perbesaran 40 kali.

- **Penilaian Kerusakan Jaringan dengan Pewarnaan HE**

Setelah tungkai tikus dipotong, dilanjutkan dengan pembuatan preparat PA dengan langkah-langkah berikut:

1. Deparafinisasi preparat yang telah kering dalam *xylol* sebanyak 2 kali (masing-masing selama 5 menit).
2. Masukkan ke dalam alkohol 96% sebanyak 2 kali (masing-masing selama 5 menit).  
Lalu cuci dengan air mengalir sampai alkohol hilang.
3. Masukkan ke dalam larutan *hematoxylin* selama 5-10 menit. Lalu cuci dengan air mengalir selama 10 menit.
4. Celupkan ke dalam larutan HCl 0.4% sebanyak 3 kali celup untuk dekolorisasi. Lalu cuci kembali dengan air mengalir selama 10 menit.
5. Masukkan ke dalam larutan *lithium carbonat* selama 30 detik lalu cuci dengan air mengalir selama 10 menit.

6. Masukkan ke dalam larutan *eosin* selama 1-2 menit, lalu cuci dengan air mengalir selama 10 menit.
7. Masukkan ke dalam larutan *ethanol* selama 15 detik sebanyak 3 kali dan selama 1 menit sebanyak 2 kali.
8. Rendam ke dalam larutan *xylol* selama 5 menit sebanyak 3 kali.
9. Tetesi dengan *entelan* dan tutup dengan *deck glass*.
10. Preparat dibaca di bawah mikroskop cahaya dengan perbesaran 40 kali
11. Hitung skor kerusakan otot skelet dengan menggunakan Tabel 4.

### LAMPIRAN 3. Dokumentasi

