REPUBLIK INDONESIA
KEMENTERIAN HUKUM DAN HAK ASASI MANUSIA

SURAT PENCATATAN CIPTAAN

Dalam rangka pelindungan ciptaan di bidang ilmu pengetahuan, seni dan sastra berdasarkan Undang-Undang Nomor 28 Tahun 2014 tentang Hak Cipta, dengan ini menerangkan:

Nomor dan tanggal permohonan	:	EC002024207543, 17 Oktober 2024
Pencipta		
Nama	:	**Dr. dr. Siufui Hendrawan, M.Biomed**
Alamat	:	Jl. Letjen S. Parman No. 1, Universitas Tarumanagara, Fakultas Kedokteran, Grogol Petamburan, Jakarta Barat, DKI Jakarta, 11440
Kewarganegaraan	:	Indonesia
Pemegang Hak Cipta		
Nama	:	**Dr. dr. Siufui Hendrawan, M.Biomed**
Alamat	:	Jl. Letjen S. Parman No. 1, Universitas Tarumanagara, Fakultas Kedokteran, Grogol Petamburan, Jakarta Barat, Dki Jakarta 11440
Kewarganegaraan	:	Indonesia
Jenis Ciptaan	:	**Buku Panduan/Petunjuk**
Judul Ciptaan	:	**Protein Isolation From Rat Liver Tissue (Cryopreserved Organ)**
Tanggal dan tempat diumumkan untuk pertama kali di wilayah Indonesia atau di luar wilayah Indonesia	:	17 Oktober 2024, di Jakarta Barat
Jangka waktu pelindungan	:	Berlaku selama hidup Pencipta dan terus berlangsung selama 70 (tujuh puluh) tahun setelah Pencipta meninggal dunia, terhitung mulai tanggal 1 Januari tahun berikutnya.
Nomor pencatatan	:	000779975

adalah benar berdasarkan keterangan yang diberikan oleh Pemohon.
Surat Pencatatan Hak Cipta atau produk Hak terkait ini sesuai dengan Pasal 72 Undang-Undang Nomor 28 Tahun 2014 tentang Hak Cipta.

a.n. MENTERI HUKUM DAN HAK ASASI MANUSIA
DIREKTUR JENDERAL KEKAYAAN INTELEKTUAL
u.b
Direktur Hak Cipta dan Desain Industri

IGNATIUS M.T. SILALAHI
NIP. 196812301996031001

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TARUMANAGARA HUMAN CELL TECHNOLOGY (THCT) LABORATORY
FACULTY OF MEDICINE, TARUMANAGARA UNIVERSITY
Jl. Letjen S. Parman No. 1; Jakarta 11440 INDONESIA
Phone. +62 21 5696 3254, Fax. +62 21 5696 7325, Email. thctlab11@gmail.com

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1. Liver Tissue Procurement and Storage

- During necropsy, the liver organ is cut into smaller tissue sections.
- Part of liver section is weighed and separated into a new sterile tube.

  Note: For protein isolation, usually only ±0.1 g of liver section is required for each 0.5-1 mL of lysis buffer cocktail.
- The organ is immediately placed in a cool box containing ice prior to storage.
- For long-term storage, the organ could be stored in deep freezer at -80°C.

2. Tissue Pulverization[1,2]

All process should be performed on ice block or at 4°C

- RIPA/PI cocktail is prepared by adding 10 µL protease inhibitor (PI; MCE Protease Inhibitor Cocktail #HY-K0010) to every 1 mL of RIPA lysis buffer (Elabscience #E-BC-R327) in a sterile microcentrifuge tube.

  Note: Pre-calculate the volume of buffer cocktail required for all samples.

  Each 0.1 g of tissue sample could be added with 0.5 – 1 mL lysis buffer (1:5 – 1:10 ratio), adjusted according to preferred experimental requirements. The RIPA/PI cocktail should be prepared freshly, right before adding into the sample. Keep the cocktail on cooling block or place it in -20°C while waiting.
- Mortar, pestle, and spatula are precooled with liquid nitrogen (LN) by carefully pouring the LN into the mortar and swirling the LN slowly using the pestle and spatula, until the LN evaporates thoroughly. This step is performed 2-3 times.

  Note: Clean all the utensils thoroughly with 70% alcohol beforehand.
- Adequate amount of LN is poured into the cold mortar, followed by soaking the frozen tissue sample in LN.
- The tissue was ground immediately until become a fine powder; LN could be added accordingly if needed.

Note: Grinding the tissue in LN should be done carefully but also as quick as possible to prevent the temperature rising.

- RIPA/PI cocktail of 0.5 – 1 mL is added into the tissue powder, continued by grinding and mixing it again.
- The powder is scraped from all over the mortar using a cold spatula and transferred into a sterile microcentrifuge tube.

  Note: Keep the whole process in a cold condition. Place the microcentrifuge tube in a cooling block or inside a cool box containing ice.

3. Protein Isolation

- Protein sample is incubated on ice or at 4^{o}C for 20 minutes
- Sample is centrifuged at 12,000 rpm, 4^{o}C for 10 minutes.
- The supernatant is collected and transferred into a new sterile tube.

  Note: Carefully collect the supernatant without disturbing the pellet.
- Sample is then subjected to protein concentration or purity measurement. If the concentration is too high, PBS solution may be added to dilute the sample.

References

1. Elabscience Bionovation Inc. RIPA Lysis Buffer (Strong). *Product Catalog* 1–2.
2. Menggensilimu *et al.* Anti-liver fibrosis effect of total flavonoids from Scabiosa comosa Fisch. ex Roem. et Schult. on liver fibrosis in rat models and its proteomics analysis. *Ann. Palliat. Med.* **9**, 272–285 (2020).