SURAT TUGAS
Nomor: 153-R/UNTAR/PENELITIAN/XI/2025

Rektor Universitas Tarumanagara, dengan ini menugaskan kepada saudara:

SIUFUI HENDRAWAN, dr., M.Biomed., Dr.

Untuk melaksanakan kegiatan penelitian/publikasi ilmiah dengan data sebagai berikut:

Judul	:	2,4,6-Trinitrobenzenesulfonic acid-induced Colitis in Mice Animal Model
Nama Media	:	DJKI
Penerbit	:	DIREKTORAT JENDERAL KEKAYAAN INTELEKTUAL
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Demikian Surat Tugas ini dibuat, untuk dilaksanakan dengan sebaik-baiknya dan melaporkan hasil penugasan tersebut kepada Rektor Universitas Tarumanagara

20 November 2025
Rektor

Prof. Dr. Amad Sudiro,S.H., M.H., M.Kn., M.M.

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OFFICE
Jl. Letjen S. Parman No 1, Jakarta Barat 11440

PHONE
+62 21-5671 747 (Hunting)
+62 21-5695 8723 (Admission)

EMAIL
humas@untar.ac.id

WEBSITE
untar.ac.id

Untar Jakarta

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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	:	EC002025173904, 7 November 2025
Pencipta		
Nama	:	**Dr. dr. Siufui Hendrawan, M.Biomed**
Alamat	:	Letjen S. Parman St No.1, RT.6/RW.16, Tomang, Grogol petamburan, West Jakarta City, Jakarta 11440, Grogol Petamburan, Kota Adm. Jakarta Barat, DKI Jakarta, 11440
Kewarganegaraan	:	Indonesia
Pemegang Hak Cipta		
Nama	:	**Dr. dr. Siufui Hendrawan, M.Biomed**
Alamat	:	Letjen S. Parman St No.1, RT.6/RW.16, Tomang, Grogol petamburan, West Jakarta City, Jakarta 11440, Grogol Petamburan, Kota Adm. Jakarta Barat, DKI Jakarta, 11440
Kewarganegaraan	:	Indonesia
Jenis Ciptaan	:	**Buku Panduan/Petunjuk**
Judul Ciptaan	:	**2,4,6-Trinitrobenzenesulfonic acid-induced Colitis in Mice Animal Model**
Tanggal dan tempat diumumkan untuk pertama kali di wilayah Indonesia atau di luar wilayah Indonesia	:	7 November 2025, di Kota Adm. 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	:	001014164

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
DIREKTUR JENDERAL KEKAYAAN INTELEKTUAL
u.b
Direktur Hak Cipta dan Desain Industri

Agung Damarsasongko,SH.,MH.
NIP. 196912261994031001

Balai Besar Sertifikasi Elektronik

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1. Dalam hal pemohon memberikan keterangan tidak sesuai dengan surat pernyataan, Menteri berwenang untuk mencabut surat pencatatan permohonan.
2. Surat Pencatatan ini telah disegel secara elektronik menggunakan segel elektronik yang ditertibkan oleh Balai Besar Sertifikasi Elektronik, Badan Siber dan Sandi Negara.
3. Surat Pencatatan ini dapat dibuktikan keasliannya dengan memindai kode QR pada dokumen ini dan informasi akan ditampilkan dalam browser.

Equipment:

1. Biosafety Cabinet Class IIA (BSC)
2. Isoflurane Anesthesia Machine
3. Analytical balance
4. Refrigerator 4ºC
5. Micropipette
6. Heating pad
7. Ruler
8. Marker

Material:

1. 2,4,6-Trinitrobenzenesulfonic acid (TNBS) (5% w/v; Sigma-Aldrich #P2297, USA)
2. Ethanol absolute (Merck #1.00983, Germany)
3. Isoflurane (Mersi, Indonesia)
4. Ketamine (Ket-A-100®, Agrovet Market, Peru)
5. Xylazine (Xyla, Interchemie #IX2, Netherlands)

Consumable:

1. Folley catheter 4FG
2. Syringe 1 cc
3. 15 mL conical tube
4. Sterile tips
5. Aluminium foil
6. Nitrile gloves
7. Surgical mask
8. 70% alcohol spray

➢ **Development of 2,4,6-Trinitrobenzenesulfonic acid (TNBS)-induced colitis in mice:**

- Mice are maintained a weight of 30-35 grams, caged in pairs and housed in a controlled environment (23°C ± 3°C, 30-70% humidity, and a 12:12 h light:dark cycle).
- Mice are rendered colitis by single intrarectal administration of Trinitrobenzenesulfonic acid (TNBS) at a dose of 120-130 mg/kg body weight (BW), following the procedure bellow:
  - Mice are fasted for 24 hour prior to TNBS administration, with free access to water.

- On the day of induction, each mice is weighed to ensure accurate calculation of the TNBS dosage.
- The injection volume is calculated individually to achieve the target dose of 120 – 130 mg/kg BW.
- A 15 mL conical tube is prepared for mixing the TNBS solution and wrapped in aluminium foil to protect the solution from light.
- A 5% (w/v) TNBS stock solution is freshly mixed with absolute ethanol at a 1:1 (v/v) ratio approximately 1 hour before injection. All preparation steps are performed under sterile conditions in a biosafety cabinet.
- The prepared TNBS solution is loaded into 1 mL syringes according to the calculated dose and connected to a 4FG foley catheter.
- Mice are anesthetized with isoflurane inhalation (5% isoflurane for induction; 1-3% for maintenance).
- The TNBS solution is aseptically and slowly injected into the colon. After injection, the mice is positioned head-down for approximately 1 minute to facilitate the distribution of the solution within the colon.

- Mice are monitored regularly for sign of distress or pain.
- If necessary, analgesia is provided using tramadol (10 – 40 mg/kg BW) to alleviate pain.

➢ **Colitis confirmation**

- After induction process completed, colitis is confirmed by assessing the scoring Disease Activity Index (DAI)[1].
- At the study endpoint, mice are anesthetized by intraperitoneal injection of ketamine (100 – 150 mg/kg BW) and xylazine (50 – 100 mg/kg BW). Euthanasia is performed by cervical dislocation after the mice are deeply anesthetized.
- The colon, including the rectum, is collected for length measurement and macroscopic observation. The colon tissue is divided into two portion: one for inflammatory marker analysis and the other for histological specimens. For histological analysis, the colon is fixed in 10% Neutral Buffered Formalin (NBF), stained with Hematoxylin & Eosin (H&E), and Immunohistochemistry to evaluate inflammatory changes in the colon.
- The degree of inflammation is determined by the scoring criteria described bellow[2], and the quantification of positive protein expression (e.g., tight junction proteins) is measured using Image J software[3].

Score	Inflammatory infiltrate	Goblet cell loss	Crypt density	Crypt hyperplasia	Muscle thickening	Submucosal inflammation	Crypt abcess	Ulceration
0	none	none	normal	none	none	none	absent	absent
1	increased presence of inflammatory cells	<10%	decreased by <10%	slight increase in crypt length	slight	individual cells		
2	infiltrates also in submucosa	10–50%	decreased by 10–50%	2–3-fold increase in crypt length	strong	infiltrate[s]		
3	transmural	>50%	decreased by >50%	>3-fold increase in crypt length	excessive	large infiltrate[s]	present	present

- Inflammatory marker analysis is performed by isolating proteins from colon tissue, followed by examination of inflammatory markers, including tumor necrosis factor-alpha (TNF-α), interleukin-10 (IL-10), myeloperoxidase (MPO), bicinchoninic acid (BCA), and superoxide dismutase (SOD).

References:

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3. Qu Y, Li X, Xu F, Zhao S, Wu X, Wang Y, et al. Kaempferol Alleviates Murine Experimental Colitis by Restoring Gut Microbiota and Inhibiting the LPS-TLR4-NF-κB Axis. Front Immunol. 2021 Jul 22;12.