Annex 1 to the Request for Bid **– Description of the Subject of the Contract**
Procedure No.: SZID.272.232.2025

DESCRIPTION OF THE SUBJECT OF THE CONTRACT

I. Detailed description of the subject of the contract:

The scope of trials being commissioned includes:

A. the conduct of pre-clinical trials to the following extent:

1. Cytokine Release Assay:
Test products will be tested in 5 dilutions (triplicates) in human and murine blood. After 14 and 24 hours the cytokine level of IFNalpha, IFNgamma, IL6 and TNFalpha will be analyzed with LegendPlex Assay.
2. Stability Testing:
Test products will be diluted in human plasma and buffer and will be stored at 2 to 8°C, 22°C and 37°C for 24h, 72h and 168h. After incubation time A549 cells will be transfected with each sample (triplicates) and cell culture supernatant will be collected at 6h, 24h and 48h. The concentration of EPO in the supernatant will be analyzed by ELISA.
3. Expression kinetics in vivo:
Adult female BALB/C mice will receive the test compounds intravenously via the tail vein. Three additional animals will serve as control. At the time points 6h, 24h and 48h three animals per group will be exsanguinated and serum will be prepared. The concentration of EPO in the serum will be analyzed by ELISA (in duplicates).

B. the preparation of a report containing protocols and results of conducted trials:

Additional information:
The Contracting Party shall deliver to the Contractor 40 µg of circRNA encapsulated in LNP with the following parameters:

Parameter	**Circular RNA (circRNA)**	**Linear RNA (mRNA)**
Purity	>80% circRNA content	>95% full-length mRNA
Integrity (RIN)	RIN > 8	
Conformation Topology Verification	PAGE analysis	PAGE analysis
dsRNA Content	Minimal to none	<1-2%
Encapsulation Efficiency (%)	>80% in LNPs	
Particle Size (nm)	50-150 nm	
Zeta Potential (mV)	-10 to +10	
RNA Yield and Concentration	>1 mg/mL	
Template Verification	Verified by sequencing	
Endotoxin Levels	<0.1 EU/µg	
Residual DNA Content	<10 ng/mg RNA	
Functional Testing	Translation in vitro	

Optical Purity (A260/280)	~2.0
UV Absorbance (A260/230)	>1.8

C. Disposal of the material remaining after the Subject of the Contract has been completed.

II. Completion date:

Report delivery deadline (to be delivered via electronic mail to the e-mail address specified in the Contract): within a maximum period of 120 days from the date on which the Contractor acknowledges receipt of material to be tested.

III. <u>Service</u> requirements:

- Łukasiewicz - PORT does not allow the Contractor to publicize the fact of cooperation with Łukasiewicz - PORT;
- The Contracting Party may require proof of compliance with the requirements of the procedure covered by the statements;

IV. Description of the subject covered in particular by professional secrecy:

The contract concerned is implemented by Łukasiewicz - PORT, as the managing entity of the Virtual Research Institute (WIB) Programme referred to in the Act on Supporting Scientific Activity from the Polish Science Fund. Łukasiewicz – PORT is responsible for the protection and commercialization of intellectual property rights to the research findings developed by Research Teams financed by the funds from the Polish Science Fund.