



<b>SOP Name</b>	Cellular immunity Assay (CI)
<b>SOP Identifier</b>	Lab008 Cellular immunity assay
<b>Edition</b>	Version 1
<b>Effective Date</b>	31/03/2022
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### 1. SCOPE:

This SOP applies to all staff, visitors, researchers, and research staff working in VACCELERATE and affiliated labs.

### 2. PURPOSE:

This SOP outlines cellular immunity assay for detection of CXCL10 by qPCR.

### 3. POLICY

VACCELERATE works within the guidelines and regulations of the EU CT Directive 2001/20/EC, GCP Commission Directive 2005/28/EC, ICH/GCP and with all other local and international applicable regulatory requirements.

### 4. ROLES AND RESPONSIBILITIES

VACCELERATE and affiliated laboratory staff carrying out this assay are responsible for its accurate and safe implementation.

### 5. DEFINITIONS

### 6. RELATED DOCUMENTS

### 7. PROCEDURES

#### 7.1. Samples to be collected and handlings to be performed

For all patients, LiHep will be collected at V1 and V2. If informed consent is given for biobanking, in all visits serum should be collected as well.

**For patients that gave informed consent for collection of PBMC, 4 additional EDTA-tubes should be collected.**

*Table 1: Summary of samples that should be collected and their purpose*

Sample	Purpose/Preparation for Test
LiHep WB	For qPCR preincubation (qPCR to be done at central lab)

*Table 2: Trial samples to be collected per time point according to clinical protocol V05\_0 (part B)*

Sample type	Procedure/test	STUDY				
		V1(screening, enrolment, baseline and 4th dose)	V2 (immune response evaluation)	V3 (follow-up)	V4 (follow-up)	V5 (end of study)
			14 ±2 days after 4th dose	3 months ± 3 days after 4th dose	6 months ± 3 days after 4th dose	12 months ± 3 days after 4th dose
1x LiHepWB	qPCR	X	x			





<sup>a</sup>: only for patients with consent for PBMC cryopreservation. The Central Lab will provide 4 extra EDTA tubes and PBMC cryopreservation tubes in a separate bag in the sample kit.

## 7.2. Overview of study supplies

- a. The Hyris XTACT(SCV3) kit qPCR preincubation kits are provided through a separate transport organised by Hyris S.r.l.
- b. N.B: Sampling collection materials such as needles, Esmarch bandage, etc. are not provided. You should use your own sampling materials in order to collect blood into the provided sample tubes.

## Description of Study Sample Material

*Table 3: Description of provided material and for which sample it needs to be used*

Sample	Material provided for sample collection	Material provided for sample processing/storage
<b>Blood (Lithium Heparin)</b>	 1x 4 ml Lithium heparin tube	 4 sterile Eppendorf tubes   Breathable seal   4x 2 ml cryovial tubes with green top

### 7.2.1. Storage conditions and expiry date of unused study materials

#### 7.2.1.1. Storage conditions

- Sample kits should be stored at room temperature and protected from direct sunlight.
- Storage conditions XTACT(SCV3) Hyris qPCR preincubation kit, see instructions of the manufacturer.

#### 7.2.1.2. Expiry dates

- Expiry dates are clearly marked on the label that is placed on the outside of the collection kit bag. The expiry date is always reflective of the tube that expires the earliest. The kit is good to the last day of the month displayed on the label.
- Expiry dates of the XTACT(SCV3) Hyris qPCR preincubation kit will be marked on the kit.

### 7.3. Detailed sample collection instructions

When a patient is enrolled into the study, the principal investigator or delegate will allocate a subject ID number to the enrolled patient. Blood sampling from each enrolled patient is due at various timepoints.

#### 7.3.1. LiHep WB for qPCR preincubation

- a) Label the lithium heparin tube with the LiHep WB label provided with the initial supplies after confirming that the participant study ID and study time point are correct and match the label on the sample requisition form.
- b) Draw the participant's blood into the lithium heparin tube.
- c) Gently invert the tube 180° and back 5-6 times.
- d) Place the tube back in the sample kit along with any of the participant's other specimens for that day, unused labels and left-over sampling material.
- e) Store the samples at room temperature until further processing. **Samples should be processed within 6 hrs.**

### 7.4. Detailed sample processing procedures

After sample collection, the sample kit can contain the following samples:

- LiHep WB

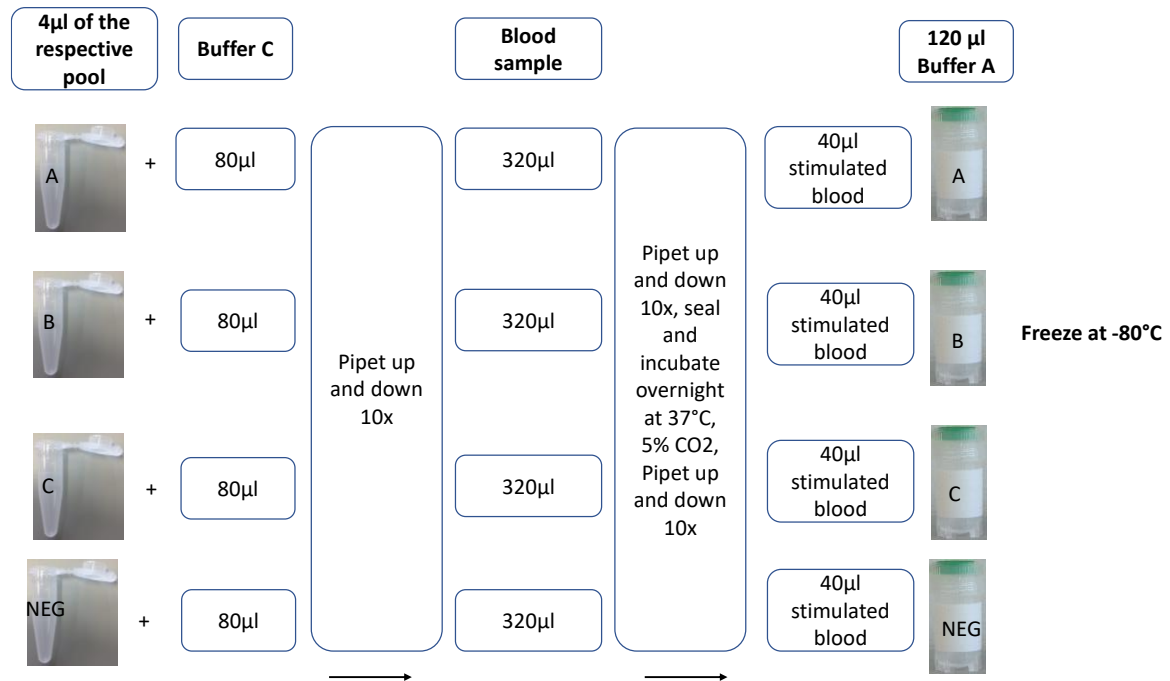
#### 7.4.1. LiHep WB for qPCR preincubation

1. Necessary reagents for the preincubation will be directly provided by the Hyris company.
2. All steps should be performed under a biosafety level 2 hood.
3. Upon first use of the XTACT(SCV3) kit, dilute Pool A, Pool B, Pool C and NEG reagents with yy  $\mu$ L of Buffer R, according to the table below

Quantity tests (XX value)	$\mu$ L of Buffer R (yy)
50	176

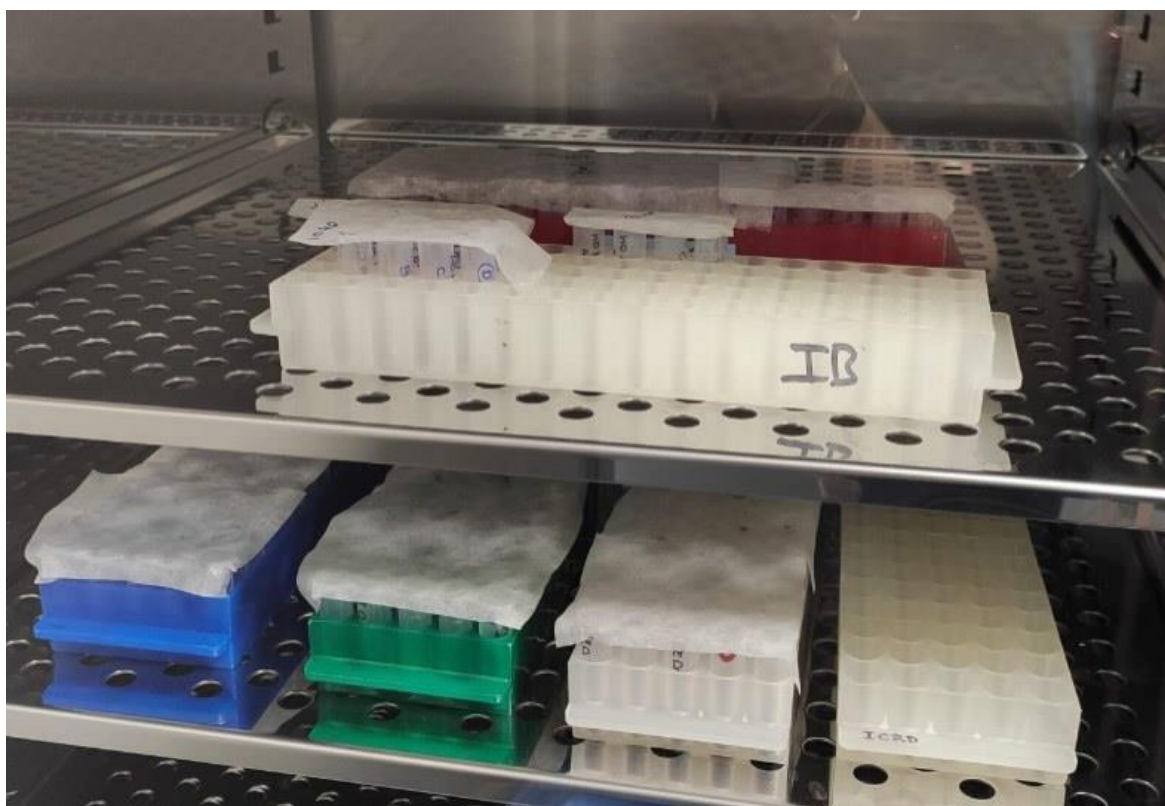
4. Mark four sterile Eppendorf tubes per sample: Tube A, Tube B, Tube C, Tube NEG
5. Remove the lid of the Eppendorf tubes with scissors.

Figure 1: qPCR preincubation scheme



6. Add 4ul of Pool A to Eppendorf tube A.
7. Add 4ul of Pool B to Eppendorf tube B.
8. Add 4μl of Pool C to Eppendorf tube C
9. Add 4ul of NEG control to Eppendorf tube NEG.
10. Add 80ul of Buffer C to Eppendorf tubes A, B, C and NEG (changing tips in between) and mix by pipetting up and own.
11. Add 320ul of whole blood to Tubes A, B, C and NEG (changing tips in between) and mix by pipetting up and down.
12. Place a breathable area seal on the top.
13. Incubate overnight at 37°C and 5% CO<sub>2</sub>.

Figure 2: Sealing of Eppendorf tubes for qPCR preincubation



14. Mix the tubes by pipetting up and down.
15. Label the green top cryotubes with the LiHep WB A, LiHep WB B, LiHep WB C and LiHep WB NEG label.
16. Dilute blood 1:4 with buffer A (e.g. 40ul blood + 120ul buffer A) in the respective green top cryotube and freeze immediately at -80°C in the qPCR storage box until shipment to the Central Lab.

## 8. REVIEW AND REVISION

## 9. DOCUMENT HISTORY

Version Number	Effective Date:	Summary of changes from previous version:	Edited by: (name and role)

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## 10. APPENDICES