HYRIS
XTACT (SCV2_P5)
Instruction for Use (IFU)

REF: HR014X050

Version 1.1

RUO
Research Use Only

Hyris Srl
Corso Garibaldi, 60 20121
Milano (MI) Italy

Ordering information
E-mail: office @hyris.net
Phone: +39.02.82951302

Info on the products
E-mail: info@hyris.net

Technical assistance
E-mail: support@hyris.net
Phone: +39.02.82951302

Hyris Srl

Corso Garibaldi, 60 20121
Milano (MI) Italy

Visit our website at
www.hyris.net

1. INTENDED USE

The **XTACT(SCV2_P5)** kit is intended for the *ex vivo / in vitro* stimulation of heparinized whole blood samples with a SARS-CoV-2 peptides pool covering the spike (S) protein and Nucleocapsid protein (NP) of SARS-CoV-2.

The **XTACT(SCV2_P5)** kit is a SARS-CoV-2 peptides pool intended to stimulate section of IFN-γ by antigen-specific T cells. *CXCL10* is a molecule expressed by monocytes in response to T cell activation. Monocytes and neutrophils are the main immune cells that increase the *CXCL10* mRNA production in response to IFN-γ.

The XTACT(SCV2_P5) is intended to be used for research, monitoring and surveillance of the epidemiological status and on the immunization condition of the COVID-19 vaccinated population and individual with experienced previous contact with SARS-CoV-2 virus population.

The stimulated sample can be measured by one of the following methods: Real-Time PCR (Hyris kit dqTACT MS ref# HR017X260, bCUBE), ELISA, ELISpot, Flow-Cytometry.
"For Research Use Only. Not for use in diagnostic procedures."

2. SUMMARY AND EXPLANATION

CXCL10 mRNA is upregulated by monocytes in response to IFN-γ secreted by antigen-specific T cells that have been stimulated with SARS-CoV-2 viral peptides in whole blood. *CXCL10* mRNA levels strongly correlate with the activation of antigen-specific T cells, serving as a "proxy" to quantify cellular immunity.
T cell reactivity can be measured directly in fresh whole blood. T cells reactive against SARS-CoV-2 peptides overexpress cytokines (e.g. IFN-γ), which are released into the plasma. This response can be measured by the direct detection of IFN-γ (ELISA or flow cytometry) or *CXCL10* (RT-PCR) . Spike-specific T lymphocytes take about 10-12 days after vaccination to develop and persist for at least more than six months after infection however, what level of protection is conferred by the presence of T lymphocyte immune response needs to be fully elucidated .

3. PRINCIPLE OF THE PROCEDURE

The **XTACT(SCV2_P5)** kit uses two synthetic peptide pools to activate cells against the Spike protein (POOL ONE) and Nucleocapsid Protein (Pool B) regions of SARS-CoV-2.
The **XTACT(SCV2_P5)** kit can be used with fresh lithium heparinized whole blood.
The effective T cell activation can be measured after incubation by cytokine production and/or specific cytokine-related gene expression (i.e. *CXCL10* mRNA). Depending on the detection technique chosen, the sample preparation may change slightly.
The **XTACT(SCV2_P5)** kit can be used in combination with the **Hyris kit dqTACT MS (HR017X260)** for the detection of *CXCL10* mRNA by RT-PCR from whole blood after RNA extraction.

4. MATERIALS PROVIDED

KIT DESCRIPTION

REF	Commercial name	Contents	Kit Size	Number of testable sample
HR014X050	XTACT(SCV2_P5)	**SARS-CoV-2 protein peptides pool** (reference strain) **Buffer R**: peptide resuspension solution **Buffer C:** cell culture buffered solution **Buffer A:** buffered salt solution containing Tween 20%	**POOL ONE SCV2**: 1x44 µL **POOL B SCV2**: 1x44 µL **POOL NEG SCV2**: 1x44 µL **BUFFER R SCV2**: 1x1000µL **BUFFER C SCV2**: 3x4500µL	50
SRXTACTSCV2015 0BARB-200	Buffer A Box	**Buffer A:** buffered salt solution containing Tween 20% ***Dilution Buffer**: buffered salt solution.*	**Buffer A: 4**x4600µL	50

Component	Cap Color	Label Color
POOL ONE SCV2	Red	Red
Pool B SCV2	Yellow	Yellow
Pool NEG SCV2	Green	Green
Buffer R SCV2	Pink	White
Buffer C SCV2	White	Blue
BUFFER A SCV2	White	White

MATERIALS REQUIRED BUT NOT PROVIDED (available separately from Hyris)

REF	Commercial name	Description	Size
Instruments			
bCUBE 2.0	bCUBE™	Miniaturized Thermal Cycler for PCR.	1 Instrument
H0001	bCUBE3	Miniaturized Thermal Cycler for PCR.	1 Instrument
Consumables			
HyCT16.01	HYRIS 16-well cartridges	Disposable 16-well cartridges for **bCUBE™** Material: Ultra-pure polypropylene and aluminum	1 pack contains 25 cartridges
HyCT36.01	HYRIS 36-well cartridges	Disposable 36 well cartridges for **bCUBE™** Material: Ultra-pure polypropylene and aluminum	1 pack contains 25 cartridges
Software			
bAPP	HYRIS bAPP™	Graphical User Interface (GUI) that works on smartphones, tablets, laptops, and PCs using any major operating system	-
Other reagents			
HR017X260	dqTACT MS	Real-Time PCR assay for the detection of CXCL10 mRNA	260 reactions
SRXTACTSCV20150BARB-200	BUFFER A Box	BUFFER A: buffered salt solution containing Tween 20%	50 Test

OTHER MATERIALS REQUIRED BUT NOT PROVIDED (available separately from Hyris)

REF	Commercial name	Description	Note
Sk.1x-Acc.01	COVID Starter kit accessories	• 3 x Precision pipettes (10µL, 100 µL, 1000 µL) • 1 x Mini centrifuge • 1 x Mini vortex	Customizable on the basis of Laboratory needs.

MATERIALS REQUIRED BUT NOT SUPPLIED

Hardware

- Incubator for cell culture at 37±1°C (with or without CO_2)
- PC, tablet, or smartphone with Google Chrome or compatible browser
- 2 to 8°C refrigerator for thawing operation (or cooler bag for transport) Freezer from -10 to -30 °C for storage
- Vortex mixer at minimum 3000 rpm
- Micropipette 200µL
- Racks for 1.5 mL microcentrifuge tubes
- PPE such as (but not limited to) respiratory protection (facemask), safety goggles, full length long (elastic) sleeved lab coat, and suitable disposable gloves.

Consumables

- Sterile DNase/Rnase free, filter tips 10µL
- Sterile Dnase/Rnase free, filter tips 100µL
- Sterile Dnase/Rnase free, filter tips 200µL
- Sterile Dnase/Rnase free, filter tips 1000µL
- 2mL Sterile, non-pyrogen, Rnase/Dnase free, polypropylene microcentrifuge tubes
- Surface decontaminants like for instance Rnase AWAY™ (Thermo Scientific Cat# 7002PK) or equivalent

5. KIT STORAGE HANDLING AND STABILITY

The **XTACT(SCV2_P5)** kit is shipped refrigerated (cold packs).

Unopened/Once opened kit

Store the reagents included in the HYRIS **XTACT(SCV2_P5)** at the following temperatures:

- **POOL ONE, POOL B** and **POOL NEG** between 2°C and 8°C
- **BUFFER C** and **BUFFER R** between 2°C and 8°C
- **BUFFER A** (SRXTACTSCV20150BARB-200) at Room Temperature (*RT*).

Allow the reagents at room temperature before use.

6. SPECIMEN COLLECTION, TRANSPORT AND STORAGE

The HYRIS **XTACT(SCV2_P5)** kit is designed to work with whole blood specimens collected with lithium heparin tubes used for plasma determination in chemistry. Proper specimen collection, storage, and transport are critical to the performance of this test. Inadequate specimen collection, handling, and/or transport may yield a false result.
Handle specimens as if they are capable of transmitting infection agents.
The HYRIS **XTACT(SCV2_P5)** kit has been validated on the following matrix: whole blood sample.

Table 1 reports the compatible collection and transport systems validated on the HYRIS **XTACT(SCV2_P5)** kit.

Compatible collection and transport system	Cap Color	Tube size	Volume (mL)	Ref #
Lithium heparin sterile single blood collection tube (Vacutainer® BD)	Green	13x100	6.0	368886

Table 1 – list of all validated collection methods

The **XTACT(SCV2_P5)** kit has been validated with the lithium heparin tube Vacutainer® BD REF# 368886.

WARNING: Tubes used for blood collection must be inverted several times immediately after collection and before processing. Insufficient mixing at this step will negatively impact sample quality.

7. SPECIMEN STABILITY

Whole blood is stable prior the stimulation at room temperature up to 6-8 hours after collection. Whole blood must be stimulated with the products of the family XTACT within 6-8 hours from collection.
Whole blood samples must be stimulated prior to the analysis with the **bKIT™ dqTACT MS**.

Long storage of stimulated sample for direct amplification

After the overnight incubation, *the recommended storage of stimulated samples, diluted in Buffer A, is -80 °C for 1 month.*

Long storage of RNA extracted from stimulated sample

After the overnight incubation *the extracted RNA should be stored at -80 °C for 3 months*[10,11,12]
Follow the Instruction For Use of the RNA extraction system selected for the RNA extraction.

8. WARNINGS AND PRECAUTIONS

- For Research Use Only (RUO);
- For Professional User;
- Wear personal protective equipment, such as (but not limited to) gloves, and lab coats when handling kit reagents and equipment. Wash hands thoroughly when finished performing the test;
- Do not pipette by mouth;
- Do not smoke, drink, eat, handle contact lenses, or apply make-up in areas where kit reagents and/or human specimens are being used;
- Dispose of unused kit reagents and human specimens as hazardous waste for incineration according to local, state, and federal regulations;

- Treat all human specimens and all consumables that have come into contact with the samples included the cartridge as capable of transmitting infectious agents;
- Contamination of patient specimens or reagents can produce erroneous results. Use good laboratory practices and control workflow;
- Only use the protocol described in this insert. Deviations from the protocol or the use of times or temperatures other than those specified may give erroneous results;
- Assay setup should be performed at room temperature (approximate range 18 to 25°C);
- Do not use kits or reagents beyond their expiration dates;
- Do not use the kits or regents if are received with primary or secondary packaging visually compromised or in warm conditions and contact the Hyris support (support.hyris.net) immediately;
- Use calibrated micro pipettes to transfer sample and reagents;
- Avoid touching the adhesive side of the aluminum foil that will be in contact with the HYRIS **bCUBE™** cartridge;
- Protect reagents from direct sources of light;
- Safety Data Sheets (SDS) are provided for each reagent upon request.

9. OPERATING PROCEDURE

The following procedures are recommended for the entire workflow with the XTACT (SCV2_P5).

WARNING: clean all the surfaces and instruments, including the external side of HYRIS bCUBE™, before and after the analytical procedure with sodium hypochlorite (0.5%) wipes.

PEPTIDE POOLS PREPARATION

a. Let the reagent reach room temperature before the use (18°C and 25°C);
b. thoroughly mix the tubes containing POOL ONE SCV2, POOL B SCV2and POOL NEG SCV2, and briefly spin them to collect the solution at the bottom;
c. resuspend POOL ONE, POOL B and POOL NEG with the BUFFER R included into the **XTACT(SCV2_P5)** kit **only at the first use** according with the table below: add 176µL of BUFFER R to the POOL ONE, POOL B, POOL NEG and mix well.

Pool of peptides	Buffer R volume
POOL ONE SCV2	176µL
POOL B SCV2	176µL
POOL Neg SCV2	176µL

SAMPLE STIMULATION

Before activating the samples, clean all instruments and work surfaces.

a. For each sample prepare 3 sterile polypropylene tubes and label them as follows:
   i. "POOL ONE" on the first tube;
   ii. "POOL B" on the second tube;
   iii. "POOL NEG" on the third tube;
   iv. Tag each tube with the sample ID;
b. Add 80 µL of Buffer C to all tubes ("POOL ONE" and "POOL B" and "POOL NEG" labeled tubes) of each sample;
c. Add 4 µL of POOL ONE to the "POOL ONE" tube of each sample and discard the tip;
d. Add 4 µL of POOL B to the "POOL B" tube of each sample and discard the tip;
e. Add 4 µL of POOL NEG to the "Pool NEG" tube of each sample and discard the tip;
f. Add 320 µL of whole blood to all the tubes for each sample ("POOL ONE" and "POOL B" and "POOL NEG" tubes). Change tips between tubes;
g. Gently mix the blood 2-3 times with the 1000µL micropipette (keeping the volume at 320µL) changing tips between tubes.

Table 2 summarizes the sample activation workflow (how to manage specimens, tubes, and reagents).

WARNING: be sure to have added the pool of peptides to the bottom of the tubes prior the blood addition. Blood, peptides and buffer C should be well mixed at the bottom of the tube before the O/N incubation.

Specimen ID	Sample Pool tube	Pool of peptides	Buffer C (µL)	Whole blood (µL)	Final volume into the 1.5 mL tube (µL)
1	1 POOL ONE	4 µL of POOL ONE	80	320	404
	1 POOL B	4 µL of POOL B	80	320	404
	1 POOL NEG	4 µL POOL NEG	80	320	404
2	2 POOL ONE	4 µL of POOL ONE	80	320	404
	2 POOL B	4 µL of POOL B	80	320	404
	2 POOL NEG	4 µL Pool NEG	80	320	404
...	....	...	...	....	...
n	n POOL ONE	4 µL of POOL ONE	80	320	404
	n POOL B	4 µL of POOL B	80	320	404
	N POOL NEG	4 µL Pool NEG	80	320	404

Table 2 – Sample preparation workflow

SAMPLE INCUBATION

a. Keep the polypropylene cap loose to allow air exchange in case incubation takes place in a cell culture incubator. If a heat block is used, be sure to close the cap of the tubes;
b. Incubate tubes overnight tubes at +37±1°C (time of incubation: from 12h to 18h) *either with or without 5% CO_2.*

SAMPLE PREPARATION

Stimulated samples may be used for direct amplification and detection or could be used after RNA extraction on bCUBE or CFX instrument. Recommended interpretation threshold has different value based on the sample preparation protocol that has been used.

WARNING: Whether the sample stimulation result is measured by one of the following methods: ELISA, ELISpot, Flow-*Cytometry, refer to the manufacturer Instruction For Use.*

BUFFER A DILUTION FOR DIRECT AMPLIFICATION ONLY

This procedure is recommended for the direct amplification workflow of stimulated samples

a. After the overnight incubation, vortex the tubes for 10 seconds at 3200 rpm;
b. For each sample take 3 new sterile microcentrifuge tubes and label them as follows:
   i. "POOL ONE" on the first tube;
   ii. "POOL B" on the second tube;
   iii. "POOL NEG" on the third tube;
   iv. Report the sample ID on all tubes;
c. *Add 40 µL of stimulated blood and 120 µL of buffer A to each tube ("POOL ONE" and "POOL B" and "POOL NEG" labeled tubes) changing tips between tubes;*
d. Mix well the tubes with a vortex at 3000 rpm for 10s;
e. Process immediately with the **bKIT™ dqTACT MS** (see the IFU of the HYRIS **bKIT™ dqTACT MS ref # HR017X260)**.

WARNING: If immediate processing is not possible, freeze the treated samples + BUFFER A at -80°C immediately.

Table 3 summarizes the sample preparation workflow (how to manage specimens, tubes, and reagents).

Sample ID	Activated blood (µL)	Buffer A (µL)	Total volume into the 1.5 mL tube (µL)
1 POOL ONE	40	120	160
1 Pool B	40	120	160
1 Pool Neg	40	120	160
....	....	....	....
n	40	120	160

Table 3 – Sample preparation workflow

WARNING: Whether the sample stimulation result is measured by one of the following methods: ELISA, ELISpot, Flow-Cytometry, refer to the manufacturer instruction for use.

RNA EXTRACTION

This procedure is recommended for the RNA extraction from stimulated blood samples.

a. *After the O/N incubation, vortex the tubes for 10 seconds to resuspend the cells and to remove cell/plasma separation;*
b. *Proceed with the RNA extraction following the system (manual extraction or automated extraction) manufacturer's instructions for use.*
c. *Once eluted, RNA can be either used for the qPCR analysis with the* ***Hyris kit dqTACT MS (HR017X260)*** *or* ***frozen at -80°C up to 3 months*** *for later analysis.*

NOTES:
For the RNA extraction Direct-zol 96-well RNA extraction kit (Zymo Research, ref# R2054/R2056) is suggested.

10. LIMITATIONS

1. For research use only;
2. For Professional User;
3. This test is a qualitative test and does not provide quantitative value of the T-Cells responsivity;
4. Some non-reactive results may occur if the analyte is present at a lower level than the analytical sensitivity of the assay;
5. This test is qualitative and does not provide a quantitative value for T cell reactivity;
6. The test result is related to the collection, handling, delivery and storage of the specimen. Any deviation from the indicated procedure will lead to an inaccurate detection result;
7. The result depends on the accuracy of sample collection and storage
8. Using blood collection systems other than those indicated will compromise the result;
9. The use of unsuitable tools can compromise the results;
10. The use of consumable non-sterile, pyrogen, endotoxin free can compromise the result;
11. Vortex mixers that do not reach 3200rpm, as well as insufficient mixing times can affect the results of the procedure;

11. BIBLIOGRAPHY

1. Schwarz, Megan et al. Rapid, scalable assessment of SARS-CoV-2 cellular immunity by whole-blood PCR. Nature biotechnology vol. 40,11 (2022): 1680-1689. doi:10.1038/s41587-022-01347-6
2. Schwarz, Megan et al., T cell immunity is key to the pandemic endgame: How to measure and monitor it. Current research in immunology vol. 3 (2022): 215-221. doi:10.1016/j.crimmu.2022.08.004
3. Le Bert N, Clapham HE, Tan AT, Chia WN, Tham CYL, Lim JM et al., Highly functional virus-specific cellular immune response in asymptomatic SARS-CoV-2 infection. J Exp Med 2021; 218. doi:10.1084/jem.20202617
4. Kalimuddin, Shirin et al. Early T cell and binding antibody responses are associated with COVID-19 RNA vaccine efficacy onset. Med (New York, N.Y.) vol. 2,6 (2021): 682-688.e4. doi:10.1016/j.medj.2021.04.003.
5. Le Bert, Nina et al. SARS-CoV-2-specific T cell immunity in cases of COVID-19 and SARS, and uninfected controls. Nature vol. 584,7821 (2020): 457-462. doi:10.1038/s41586-020-2550-z
6. Tan, Anthony T et al. Early induction of functional SARS-CoV-2-specific T cells associates with rapid viral clearance and mild disease in COVID-19 patients. Cell reports vol. 34,6 (2021): 108728. doi:10.1016/j.celrep.2021.108728
7. Tan, Anthony T et al. Rapid measurement of SARS-CoV-2 spike T cells in whole blood from vaccinated and naturally infected individuals. The Journal of clinical investigation vol. 131,17 (2021): e152379. doi:10.1172/JCI152379
8. Coronaviridae Study Group of the International Committee on Taxonomy of Viruses. The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. Nat Microbiol. 2020; 5(4): 536-44.
9. Lozano-Ojalvo, Daniel et al. Differential effects of the second SARS-CoV-2 mRNA vaccine dose on T cell immunity in naive and COVID-19 recovered individuals. Cell reports vol. 36,8 (2021): 109570. doi:10.1016/j.celrep.2021.109570.
10. Kellman, B.P., Baghdassarian, H.M., Pramparo, T. et al. Multiple freeze-thaw cycles lead to a loss of consistency in poly(A)-enriched RNA sequencing. BMC Genomics 22, 69 (2021). https://doi.org/10.1186/s12864-021-07381-z
11. Seelenfreund, Eric et al. "Long term storage of dry versus frozen RNA for next generation molecular studies." PloS one vol. 9,11 e111827. 7 Nov. 2014, doi:10.1371/journal.pone.0111827
12. *Thermo Fisher Scientific, technical bulletin # 159: Working with RNA;*

12. SYMBOLS DESCRIPTION

SYMBOL	DESCRIPTION
	LOT
	Catalogue number
	Expiration date
	Manufacturer
	Refer to the instructions for Use/functioning.
	Content sufficient for <n> test
	Temperature range recommended
	Do not use if the packaging is broken

Released: May 2023

The **XTACT(SCV2_P5)** have been internally tested by our quality control. Any responsibility is waived for any off-label use.
HYRIS bCUBE™, HYRIS bAPP™ and HYRIS Hyris kit are a trademark of HYRIS S.r.l.