HYRIS XTACT(SCV4)

Instruction for Use (IFU)

REF: R-XTACT(SCV4)-50

Version 1.3

RUO

Research Use Only

INTENDED USE

The **XTACT(SCV4)** 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, Nucleocapsid protein (NP) and regions of the protein S mutated into the omicron variant of SARS-CoV-2 virus.

The **XTACT(SCV4)** 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(SCV4)** 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 bKIT™ dqTACT MS, bCUBE], ELISA, ELISpot, Flow-Cytometry.
Not to be used for diagnostic purposes.
For Research Use Only.

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 [1].
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) [1]. Spike-specific T lymphocytes take about 10-12 days after vaccination to develop [11] 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 [11].

PRINCIPLE OF THE PROCEDURE

XTACT(SCV4), uses three synthetic peptide pools to activate cells against the Spike protein (POOL ONE), Nucleocapsid Protein (POOL B) and omicron variant mutations (POOL C) regions of SARS-CoV-2.
XTACT(SCV4) 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 (ELISA or flow cytometry) or *CXCL10* (RT-PCR) the sample preparation may vary slightly.
The **XTACT(SCV4)** kit can be used in combination with the **bKIT™ dqTACT MS** for the detection of *CXCL10* mRNA by RT-PCR either directly from whole blood or after RNA extraction.

MATERIALS PROVIDED

KIT DESCRIPTION

REF	Commercial name	Contents	Kit Size	Number of testable sample
R-XTACT(SCV4)-50	XTACT(SCV4)	**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 C SCV2**: 1x44 µL **POOL NEG SCV2**: 1x44 µL **BUFFER R SCV2**: 1x1000µL **BUFFER C SCV2**: 3x4500µL	50
SRXTACTSCV20150 BARB-200	Buffer A Box	**Buffer A:** buffered salt solution containing Tween 20%	**Buffer A SCV2: 4**x4600µL	50

Component	Label Color
POOL ONE SCV2	Red
POOL B SCV2	Yellow
POOL C SCV2	Blue
POOL NEG SCV2	Green
BUFFER R SCV2	White
BUFFER C SCV2	Blue

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			
HR017X300	dqTACT MS	Real-Time PCR assay for the detection of *cxcl10* mRNA	300 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, nonpyrogen, RNAse DNAse free, polypropylene microcentrifuge tubes
- Surface decontaminants like for instance RNAse AWAY™ (Thermo Scientific Cat# 7002PK) or equivalent

KIT STORAGE HANDLING AND STABILITY

The **XTACT(SCV4)** kit is shipped refrigerated (cold pakcs).

Unopened/Once opened kit

Store the reagents included in the HYRIS **XTACT(SCV4)** at the following temperatures:

- **POOL ONE SCV2, POOL B SCV2, POOL C SCV2 and POOL NEG SCV2** between 2°C and 8°C
- **BUFFER C and BUFFER R** between 2°C and 8°C

BUFFER A (SRXTACTSCV20150BARB-200) at Room Temperature (TA).
Allow the reagents at room temperature before the use.

SPECIMEN COLLECTION, TRANSPORT AND STORAGE

The HYRIS **XTACT(SCV4)** 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(SCV4)**
kit has been validated on the following matrix: whole blood sample.

Table 1 reports the compatible collection and transport systems validated on the HYRIS **bKIT™ XTACT(SCV4)**.

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

Table 1 - list of all validated collection methods

The **XTACT(SCV4)** 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.

Specimen stability

Whole blood must be treated with the **HYRIS XTACT(SCV4)** within 6-8 hours from collection. Whole blood should be kept at room temperature up to 6-8 hours after collection.
Do not freeze the blood or keep it at 4°C before stimulation with the **XTACT(SCV4)**.

After stimulation with **XTACT(SCV4)**, samples must be immediately analyzed and/or immediately stored at -80°C after the immediate dilution of stimulated blood with BUFFER A (see sample preparation paragraph).

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) 6mmediately
- 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;

OPERATING PROCEDURE

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

POOL PREPARATION

a. Brought to the reagents at room temperature before the use (between 18°C and 25°C)
b. Resuspend the POOL ONE SCV2, POOL B SCV2, POOL C SCV2 and POOL NEG SCV2 with the BUFFER R SCV2 included into the **XTACT(SCV4)** kit prior the first use according with the table below: add 176µL of BUFFER R SCV2 to the POOL ONE SCV2, POOL B SCV2, POOL C SCV2, POOL NEG SCV2 and mix well.

Pool of peptides	BUFFER R volume
POOL ONE SCV2	176µL
POOL B SCV2	176µL
POOL C SCV2	176µL
POOL NEG SCV2	176µL

1. SAMPLE TREATMENT

WARNING: mix well with a vortex (3000rpm) the peptides pool before the use
Before activating the samples, clean all instruments and work surfaces.

a. For each sample prepare 4 sterile polypropylene tubes and label them as follows:
   i. "POOL ONE" on the first tube.
   ii. "POOL B" on the second tube.
   iii. "POOL C" on the third tube.
   iv. "POOL NEG" on the fourth tube.
   v. Report the sample ID on all tubes.
b. Add 4 µL of POOL ONE SCV2 to the "POOL ONE" labeled tube of each sample then discard the tip
c. Add 4 µL of POOL B SCV2 to the "POOL B" labeled tube of each sample then discard the tip
d. Add 4 µL of POOL C SCV2 to the "POOL C" labeled tube of each sample then discard the tip
e. Add 4 µL of POOL NEG to the "POOL NEG" labeled tube of each sample then discard the tip
f. Add 80 µL of BUFFER C SCV2 to all tubes ("POOL ONE" and "POOL B" and "POOL C" and "POOL NEG" labeled tubes) of each sample changing tips between tubes
g. Add 320 µL of whole fresh blood to all tubes ("POOL ONE" and "POOL B" and "POOL C" and "POOL NEG" labeled tubes) changing tips between tubes
h. 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 SCV2 should be well mixed at the bottom of the tube prior to 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 C	4 µL of POOL C	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 C	4 µL of POOL C	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 C	4 µL of POOL C	80	320	404
	n POOL NEG	4 µL POOL NEG	80	320	404

Table 2 – Sample preparation workflow

2. SAMPLE INCUBATION

a. Keep the cap of the polypropylene loose to allow the air exchange.
b. Incubate tubes overnight tubes at +37±1°C (time of incubation: 12h-18h).

3. SAMPLE PREPARATION (Direct amplification Workflow)

a. After the overnight incubation, vortex the tubes for 10 seconds at 3200 rpm.
b. For each sample take 4 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 C" on the third tube.
    iv. "POOL NEG" on the fourth tube.
    v. Report the sample ID on all tubes.
c. Add 40 µL of stimulated blood to each tube ("POOL ONE" and "POOL B" and "POOL C" and "POOL NEG" labeled tubes) changing tips between tubes.
d. Add 120 µL of BUFFER A to all tubes ("POOL ONE" and "POOL B" and "POOL C" and "POOL NEG" labeled tubes) changing tips between tubes
e. Mix well the tubes with a vortex at 3000 rpm for 10s
f. Process immediately with the **bKIT™ dqTACT MS** (Refer to IFU of the HYRIS **bKIT™ dqTACT MS.**

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 SCV2	40	120	160
1 POOL B SCV2	40	120	160
1 POOL B SCV2			
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.

4. RNA EXTRACTION (Extracted RNA workflow)

g. After the overnight incubation, vortex the tubes for 10 seconds at 3200 rpm.
h. Proceed with the RNA extraction using an extraction kit validated for RNA extraction from blood (refer to the instructions for use of the extraction kit)

Once eluted, extracted RNA can be either used for the qPCR analysis with the Hyris bKIT™ dqTACT MS or frozen at -80°C up to 3 moths for later analysis. Refer to the bKIT™ dqTACT MS instructions for use

RESULT INTERPRETATIONS

Interpretation of results is automatically performed by HYRIS bAPP for samples analyzed with the **bKIT™ dqTACT MS** kit used on **HYRIS bCUBE and third party instruments (QuantStudio 5 and Bio-Rad CFX96) using HYRIS bGATE**. The "RESULTS" tab will display the final result.

In case the analysis is performed on other instruments, follow the procedure "**DATA EXPORT AND ANALYSIS**" for data export.

Table 4**Error! Reference source not found.** shows the Ct cut-off obtained by the analysis with the kit **bKIT™ dqTACT MS**

Real Time PCR Ct cutoff		
Campione	TARGET	Ct cutoff
SAMPLE	*CXCL10*	Valid for Ct ≤42
	ACTIN	Valid for Ct ≤33

Table 4 - bKIT™ dqTACT MS Ct cut-off

Using the HYRIS **bKIT™ dqTACT MS** on the **HYRIS bCUBE** with the **HYRIS bAPP**, the results are automatically calculated and expressed in terms of reactivity.

After the quality check, a valid sample is interpreted as indicated in Table 5

bKIT™ dqTACT MS RESULTS – Direct amplification workflow			
bAPP RESULTS	Threshold value Direct amplification	Threshold value *Extracted RNA*	T CELL ANTIGEN SPECIFIC REACTIVITY
NOT REACTIVE	< 0.001	< 0.05	NOT RESPONSIVE to specific-peptides pool stimulation
REACTIVE	> 0.003	> 0.05	RESPONSIVE to specific-peptides pool stimulation
BORDERLINE REACTIVITY	0.001≤x≤0.003	-	BORDERLINE RESPONSIVE to specific-peptides pool stimulation
INCONCLUSIVE	-	-	INCONCLUSIVE the *ACTIN* Ct into the stimulated sample is over the Ct Cut-off
INVALID	-	-	INVALID One of the controls DOES NOT exhibit the expected performance and the analysis CAN NOT be consider valid. Repeat the analysis

Table 5 - bKIT™ dqTACT MS interpretation table result

If the inconclusive and invalid result persist, contact Hyris technical support at support.hyris.net.

Protocol and data interpretation have been described according to Schwartz et al., 2022.

DATA EXPORT AND ANALYSIS FOR HYRIS bGATE INTERPRETATION

Bio-Rad

- Open the analysis on Biorad CFX.96 maestroclick on export button
- Select export all data sheet
- Click on the .csv button to export data into the folder bgate

Follow the procedure for data importation on Hyris bGATE specific for your use (manual loading or fully automated loading)

QuantStudio 5

- Open the analysis on the Design and Analysis software
- Click on analyze button
- Click on action button
- Click on export button, then select csv format
- Browse the folder bgate, thenk click on export

Follow the procedure for data importation on Hyris bGATE specific for your use (manual loading or fully automated loading)

DATA EXPORT AND ANALYSIS FOR MANUAL INTERPRETATION

a. Downloads the analysis Excel report file from support.hyris.net
b. Download the results interpretation file, for instance"**dqTACT for XTACT(SCV3&4) interpretation_V xx.xlsx**".
c. insert Ct values into the "Data insertion" sheet of the **interpretation** file coping the FAM and HEX Ct values of each sample from the analysis report file.
d. **Copy the FAM and HEX Ct values from the analysis report Excel sheet into the "Data insertion" tab of the dqTACT for XTACT(SCV3&4) Interpretation file.**
e. The "Interpreted Result" tab will display the final result.

Hyris Srl recommends a phased approach for the interpretation of the results with the Real Time PCR method.
Phase one, verification of the validity of the Positive and Negative controls of the test. Once this validity is established, the data can be interpreted.
Phase two, verification of the validity of the internal control value (ACTIN), an indication of the presence of sufficient cellular material for analysis. Ct values of *ACTIN* less than 33 are accepted.
Phase three, final data interpretation stage, in which the sample response to stimulation by the peptide pools is evaluated. Stimulation is evaluated by considering the Ct values of *CXCL10* and *ACTIN* obtained by treatment with the peptide pools (treated sample) and those obtained with the sample stimulated with POOL NEG SCV2 (untreated sample)
Phase four , the change of *CXCL10* in stimulated samples, by subtraction of the BASE value, on the post-vaccination immune reaction is evaluated.

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.

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SYMBOLS DESCRIPTION

SYMBOL	DESCRIPTION
	LOT
	Catalog 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

Ordering information	Technical assistance	
E-mail: office@hyris.net	E-mail: support@hyris.net	E-mail: info@hyris.net
Phone: +39.02.82951302	Phone: +39.02.82951302	

Visit our website: https://www.hyris.net

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 Hyris S.r.l.
Viale V. Lancetti 19
20158 Milano (MI), Italy