Customer Support

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Symbols Used

Symbol	Description		
RUO	This symbol marks a product as for "Research Use Only".	⚠	All warnings are marked by warning triangle with exclamation mark. Depending on their character they are supplemented with the words ATTENTION or CAUTION.
	Manufacturer	REF	Reference number
Σ	No of tests	LOT	Lot number
	Expiry date		Temperature limitation for storage. Lower and upper limits are indicated.

Revision: XRNA-RUO-RevC210219-200918

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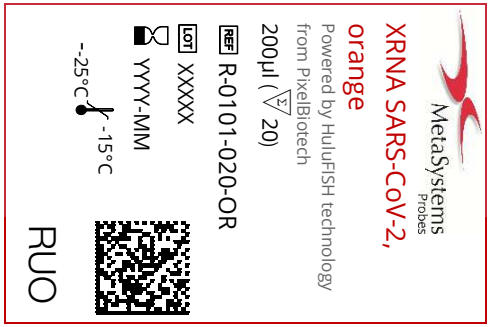

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Product	Label	Order No.	Pack Size
XRNA SARS-CoV-2, orange	orange	R-0101-020-OR	200µl

Product Description

The XRNA SARS-CoV-2 probe kit comprises 96 oligos detecting the spike glycoprotein mRNA of SARS-CoV-2 and a portion of the viral ORF1 mRNA. We are using the proprietary HuluFISH enzymatic multi fluorophore labeling technique enabling the detection of RNA at the single-cell, single-molecule level in cell and tissue samples. The probe kit is labeled in orange (Atto565).

Materials Provided

200µl of XRNA SARS-CoV-2, orange, which is sufficient for 20 tests using the recommended 10 µl probe volume per test. The recommended coverslip size is 18 x 18 mm².

Safety Instructions

All probe kits produced by MetaSystems Probes are for professional use only and should only be used by qualified and trained personnel. To ensure safe operation and reproducible results please observe the safety notices and caution signs below.

⚠	**CAUTION: Hot water bath and hot plates!**
	For denaturation and hybridization hot water baths and hot plates are used with temperatures of >37°C. Be careful not to get in direct contact with hot surfaces or liquids. Wear gloves and a lab coat. In case of contact with skin, cool immediately with cold water.
⚠	**ATTENTION: Good Laboratory Practice!**
	Use in accordance with the principles of good laboratory practice.

Storage and Handling

Probes should be stored in the dark at -20°C (±5°C).

Shipping

MetaSystems' XRNA probes are shipped at room temperature.

Materials Required but not Provided

Section 1	Section 2	Section 3
• Optional: sterile PBS or TE (TrisEDTA pH 7.4) • water, double distilled (ddH_2O) • washing buffer (2x SSC, 2 M Urea in ddH_2O) • 100 % Ethanol optional • hypotonic solution KCl 50 mM • fixative (3:1 Methanol:Acetic acid) • Pepsin (2000-2500 U/mg): 10 µg/ml in 0.01 M HCl, 37 °C • Metasystems' DAPI/antifade, Ref.-No. D-0902-500-DA	• DTT-free mycolytic agent: MetaSystems Liquilizer® • washing buffer (2x SSC, 2 M Urea in ddH_2O) • hypotonic solution KCl 50 mM • fixative (3:1 Methanol:Acetic acid) • 1x PBS • 2x SSC • Pepsin (2000-2500 U/mg): 10 µg/ml in 0.01 M HCl, 37 °C • Ethanol 70%, 80 %, 100 % • Metasystems' DAPI/antifade, • Ref.-No. D-0902-500-DA	• water, double distilled • washing buffer (2x SSC, 2 M Urea in ddH_2O) • Xylene • Ethanol 70%, 95 %, 100 % • fixative (3:1 Methanol:Acetic acid) • 4 % Formaldehyd in 1x PBS • 135 mM Glycine in 1x PBS solution • 1x PBS • 0.01 M pH 6 Sodium Citrate solution, 80°C • Pepsin (2000-2500 U/mg): 3mg/ml in 0.01 M HCl, 37°C, • Metasystems' DAPI/antifade, Ref.-No. D-0902-500-DA

Sample Preparation

General Comments

Please consider the following information as recommendations. Changes and adaptations could be necessary based on personal laboratory routine and different types of samples which are not reported in this protocol. All employed solutions and water must be RNase-free.

SECTION 1 – Cellular Samples from Swabs

Two different tools can be used for swab sampling: the actual cotton "swab" and the "brush". The use of swabs or brushes must be evaluated based on the subject (newborns or adults) and applications (clinical-diagnostic, forensic, or forensic "post-mortem"). This protocol will refer to both tools as "swab". The sample can be stored by one of the following methods:

Dry Swab: To wash and harvest the cellular sample, add just enough volume of sterile PBS or TE (TrisEDTA pH 7.4) to cover the cotton swab area. Store for 12-24 hours at 4°C.

Wet Swab: 15 ml tube with cotton swab or brush immersed in PBS w/ antibiotics (1% penicillin/streptomycin is advised).

1. Cut the far end with the swab and centrifuge at 1500 rpm **for 2 min**.
1. Carefully remove the swab using tweezers that have been previously cleaned with absolute Ethanol.
2. Centrifuge the tube at 1500 rpm **for 5 min**. Discard the supernatant, leaving around 0.5 ml.
3. Resuspend the pellet.
4. Add 4.5 ml of hypotonic solution (KCl 50 mM), carefully resuspend the pellet, and incubate at RT **for 20 min**.
5. Add 5 ml of fixative (Methanol/Acetic acid 3:1, freshly prepared) and resuspend carefully.
6. Centrifuge at 1500 rpm **for 5 min**. Discard the supernatant, leaving around 0.5 ml.
7. Resuspend the pellet.
8. Repeat steps 6-7 (1x) but remove the supernatant completely without touching the pellet.
9. Add 100 µl of fixative and resuspend the pellet.
10. Drop 15-20 µl of sample on the slide (otherwise, the fixed sample can be stored at -20°C for future use).

Before proceeding with the MetaSystems' FISH protocol, a digestion step with Pepsin **for 10 min** is recommended (see "Pepsin Digestion").

After preparing the sample from buccal/oral swabs, please proceed as follows:

Day 1: Proceed with "Hybridization".

Day 2: Proceed with "Washing Steps".

References:

1. "Potential Use Of Blood, Buccal And Urine Cells For Rapid Noninvasive Diagnosis Of Suspected Aneuploidy Using Fluorescence In Situ Hybridization (FISH)", JCDR – 2007; 1:33-38

SECTION 2 - Cellular Samples from Sputum, BAL and BAS

We strongly suggest a sample treatment with a DTT-free mucolytic agent, which is safer, easier to handle, and stable. MetaSystems Liquillizer® is a ready-to-use DTT-free mucolytic agent indicated for treatment of viscous respiratory samples such as sputum, bronchoalveolar lavage (BAL) and bronchial aspirate (BAS).

- The liquifying buffer appears odorless and is stable at room temperature up to 4 weeks.
- Sample warming is not required during incubation.
- Samples must be processed fresh, immediately after sampling.

Day 1:

1. Add Liquillizer® to the sample (equal to the amount of the sample volume).
2. Vortex until sample appears liquefied (around **30 seconds**).
3. Centrifuge the sample at 1500 rpm **for 10 min**. Carefully discard supernatant leaving only a very small volume of 1xPBS.
4. Wash 3 times with 1x PBS (centrifuge each time at 1500 rpm **for 5 min**).
5. Resuspend the pellet with residual volume.
6. Add 4.5 ml of hypotonic solution (KCl 50 mM), resuspend carefully, and incubate at RT **for 20 min**.
7. Add 5 ml of fixative (Methanol/Acetic acid 3:1, freshly prepared) and resuspend carefully.
8. Centrifuge at 1500 rpm **for 5 min**. Discard supernatant, leaving just 0.5 ml.
9. Resuspend the pellet.
10. Add once again 5 ml of fixative solution.
11. Repeat steps 9-12 (1x).
12. Centrifuge at 1500 rpm **for 5 min**. Carefully discard the whole supernatant, without touching the pellet.
13. Add 100 µl of fixative and resuspend the pellet.
14. Drop 15-20 µl of sample on the slide.
15. If you do not need to process the sample immediately, please store it at -20°C for future use.
16. Before proceeding with hybridization, a pepsin digestion (see "Pepsin Digestion") is mandatory.

Day 2: Proceed with "Washing Steps".

Pepsin Digestion (Section 1 + 2)

1. Pre-treat the slide with 2x SSC at RT **for 2 min**.
2. Digest the sample with Pepsin (10 µg/ml in 0.01 M HCl) at 37°C **for 3 -10 min**.
3. Dehydrate the sample as follows:
4. 70% Ethanol at RT **for 2 min**.
5. 80% Ethanol at RT **for 2 min**.
6. 100% Ethanol 100% at RT **for 2 min**.
7. Let air dry.

SECTION 3 – Tissue Sections

Tissue sections of 2-4 µm are recommended for optimal results.

1. Deparaffinization and rehydration of the sample (for FFPE sections)
    a. Pre-warm the tissue section at 56°C **overnight.**
    b. Immerse the slide 2 times in Xylene at RT **for 10 min each.**
    c. Immerse the slide 2 times in 100% Ethanol at RT **for 5 min each.**
    d. Immerse the slide in Methanol/Acetic acid 3:1 at RT **for 5 min**.
    e. Immerse the slide in 100% Ethanol at RT **for 3 min**.
    f. Immerse the slide in 95% Ethanol at RT **for 3 min**.
    g. Immerse the slide in 70% Ethanol at RT **for 3 min**.
    h. Immerse the slide ddH_2O at RT **for 3 min**.
    i. Go to step 2.

1a. Fixation (for cryostat sections)
    a. Fix the section by immersing the slide with 4% Formaldehyde in 1x PBS solution **for 10 min**.
    b. Block the fixation process by immersing the slide in 135 mM Glycine in 1x PBS solution **for 10 min.**
    c. Wash the slide with 1x PBS at RT **for 10 min**.
    d. Permeabilize the sample with 70% Ethanol at 4°C **overnight.**
    e. Go to step 3.

2. Sample pre-treatment
    a. Pre-treat the sample with a 0.01 M pH 6 Sodium Citrate solution at 80°C **for 45 min**.
    b. Wash the slide three times with ddH_2O for **3 min each**.
    c. Digest the sample with Pepsin (3mg/ml in 0.01 M HCl) at 37°C **for 3 min**.
    d. Digest a second time with a new freshly made Pepsin (3mg/ml in 0.01 M HCl) at 37°C **for 2 min** (between steps c. and d. the digestion quality can be evaluated at the microscope and the digestion procedure can be adjusted accordingly).

3. Proceed immediately with "Hybridization".

4. Day 2: Proceed with "Washing Steps".

Hybridization

1. Wash the sample in ddH_2O at RT **for 3 min**.
2. Incubate the sample 2 times in washing buffer (2x SSC, 2 M Urea in ddH_2O) each at RT **for 10 min**.
3. Carefully remove excess liquid from the slide, without touching the sample area (tissue paper can be used).
4. Add 10 µl of the XRNA probe directly onto the sample and cover with an 18 x 18 mm² coverslip (avoid bubbles).
5. Seal the slide with rubber cement.
6. Hybridize **for 16-18 hours** in a humid chamber at 30°C.

Washing Steps

1. Carefully remove rubber cement and coverslip.
2. Wash the slide 4 times in washing buffer (2x SSC, 2 M Urea in ddH_2O), each at RT **for 10 min**.
3. Carefully remove excess washing buffer from the slide without touching the sample area (tissue paper can be used).
    a. Not mandatory, suggested step: quickly dehydrate the sample **for 30 seconds** in 100% Ethanol.
4. Let the slide air dry.
5. Add 10 µl of DAPI/Antifade (e.g. Metasystems' DAPI/antifade, Ref.-No. D-0902-500-DA).
6. Carefully place an18 x 18 mm² cover glass on top of the DAPI/Antifade (avoid bubbles) and seal the slide.
    a. Not mandatory, suggested step: to reduce DAPI intensity, the slide can be stored at -20°C **for 20 min**.