

virotype[®] Tissue Lysis Reagent Handbook

Tissue Lysis Reagent for fast lysis of various
sample types

REF 100 ml (cat. no. SP289992)

REF 250 ml (cat. no. SP289993)



INDICAL BIOSCIENCE GmbH, Deutscher Platz 5b,
04103 Leipzig, Germany

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Kit contents

Cat. no.	SP289992	SP289993
virotype Tissue Lysis Reagent	100 ml	250 ml
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Intended use

virotype Tissue Lysis Reagent is an extraction reagent for the fast preparation of various sample types without nucleic acid extraction procedures.

The virotype Tissue Lysis Reagent is designed and has been validated for fresh or dried ear tissue sample preparation from cattle (size of ear tissue Ø 2-3 mm) and for tracheal swabs (individual and pooled samples) from chicken and turkey.

The lysis of other samples types may be possible but need to be validated by the customer.

Symbols



Legal manufacturer



Lot number



Use by date



Temperature limitations for storage



Handbook



Catalog number



Material number

Quality control

In accordance with INDICAL's ISO-certified Quality Management System, each lot of virotype Tissue Lysis Reagent is tested against predetermined specifications to ensure consistent product quality.

Storage

The virotype Tissue Lysis Reagent should be stored at -30°C to -15°C and is stable until the expiration date stated on the label. Freeze the components in aliquots if only used intermittently.

Safety information

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available from your local sales representative or by Email request under **compliance@indical.com**.

All sample residues and objects that have come into contact with samples must be decontaminated or disposed of as potentially infectious material.

Protocol 1: Lysis of ear tissue samples

Note: This protocol has only been validated for ear tissue samples from cattle (2-3mm diameter) and the subsequent downstream application using virotype BVDV RT-PCR Kit.

Procedure

1. Bring virotype Tissue Lysis Reagent to room temperature (18-25°C) before use. Calculate and take the volume needed and freeze the remaining lysis buffer.
2. Add 200 µl virotype Tissue Lysis Reagent to each ear tissue sample (Ø 2-3 mm) directly into the sampling device.
3. Close the tubes with the corresponding caps or with aluminum sealing foil, appropriate for heat incubations.
4. Incubate the samples as shown in Table 1.

Table 1. Incubation times

Step	Temperature	Volume
1	65°C ^{1, 2}	30 min
2	98°C ¹	15 min

¹ Perform incubation in drying oven or sterilizing oven. Heating blocks are recommended for well-fitting reaction tubes only.

² Incubation time can be extended up to 10 minutes.

5. Allow the sample to cool down for 5 min at 2-8°C (e.g., on ice).
6. Process lysates quickly. Lysates which will not be tested immediately can be stored up to 12 h at 2-8°C or up to 6 months at -30°C to -15°C.

Pooling

After lysis of individual ear tissue samples is completed, pool equal volumes (at least 10 µl) of the lysates and mix (e.g., pools of 20: pool 10 µl of 20 lysates respectively [200 µl total pool sample volume]).

Real-time RT-PCR

virotype Tissue Lysis Reagent has only been validated in combination with virotype BVDV RT-PCR Kit.

5 µl of each pool/ lysate is tested directly with virotype BVDV RT-PCR Kit. In rare cases, depending on sample quality, testing of lysates of individual samples can result in invalid tests as a consequence of PCR inhibition (neither FAM fluorescence nor HEX fluorescence is detected). In such cases it is recommended to mix 2.5 µl of lysate with 2.5 µl RNase-free water and to retest.

Thaw frozen lysates at room temperature and gently mix the sample by drawing the sample into a pipet tip and expelling back into the sampling device and repeating the process once or twice before use.

Protocol 2: Lysis from avian swabs

Procedure

1. Bring virotype Tissue Lysis Reagent to room temperature (18-25°C) before use. Calculate and take the volume needed and freeze the remaining lysis buffer.
2. Dilute virotype Tissue Lysis Reagent 1:5 in nuclease free-water or phosphate buffered saline. Alternatively, virotype Tissue Lysis Reagent can be used undiluted.
3. Place the swab in a suitable, disposable reaction tube (see Table 2).
4. Add virotype Tissue Lysis Reagent to each reaction tube. Ensure all swabs are completely covered by the virotype Tissue Lysis Reagent (Table 2).

Table 2. Swab types

	Single sample	Pools		
		Thin swab	Thick swab	
Reaction tube	2 ml	2 ml	15 ml round bottomed tube	
Swabs	1	2-5	2-3	4-5
Lysis buffer	500 µl	500 µl	1000 µl	1500 µl

5. Close the reaction tubes with the corresponding cap and briefly vortex.
6. Incubate the sample as shown in Table 3.

Table 3. Incubation times

Step	Temperature	Volume
1	65°C ^{1, 2}	30 min
2	98°C ¹	15 min

1 Perform incubation in drying oven or sterilizing oven. Heating blocks are recommended for well-fitting reaction tubes only.

2 Incubation time can be extended up to 10 minutes.

7. Allow the sample to cool down for 5 min at 2-8°C (e.g., on ice).
8. Process lysates quickly. Lysates which will not be tested immediately can be stored up to 5 days at 2-8°C or up to 6 months at -30°C to -15°C.

Pooling

For testing pools of up to 10 swabs, take equal volumes of the lysates after completed lysis and mix them well (at least 10 µl of each lysate).

Real-time PCR

virotype Tissue Lysis Reagent has only been validated in combination with bactotype[®] Mycoplasma Mg/Ms PCR Kit.

5 µl of each lysate/ pool is tested directly with bactotype Mycoplasma Mg/Ms PCR Kit. In rare cases, depending on sample quality, testing of lysates of individual samples can result in invalid tests as a consequence of PCR inhibition (no fluorescence is detected). In such cases it is recommended to mix 2.5 µl of lysate with 2.5 µl nuclease-free water and to retest.

Thaw frozen lysates at room temperature and briefly vortex the samples.

INDICAL offers a range of ELISA kits and real-time PCR and real-time RT-PCR kits for the detection of animal pathogens.

Visit **www.indical.com** for more information about bactotype, cadof, cattletype, flocktype, pigtype and virotype products.

For up-to-date licensing information and product-specific disclaimers, see the respective INDICAL kit handbook or user manual

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