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cattletype® Milk Prep Kit Handbook

For concentration and purification of antibodies from milk samples

REF 50 samples (cat. no. SP271906)

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Kit contents

cattletype Milk Prep Kit **Cat. no.** **Number of preparations**	**(50)** **SP271906** **50**
Precipitation Reagent, ready to use	1 x 60 ml
Neutralization Buffer, ready to use	3 x 60 ml
Matrix, ready to use	2 x 20 ml
Elution Buffer, ready to use	1 x 20 ml
Spin columns	50
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Intended use

cattletype Milk Prep is intended for the concentration and purification of antibodies from individual, pooled, and bulk milk samples. Concentration of antibodies is a tool to enhance the sensitivity of testing milk samples using immunoassays (e.g., ELISA). The cattletype Milk Prep Kit also helps to minimize non-specific reactions in individual milk samples by purification of those samples. It is recommended for pools of up to 50 milk samples or bulk milk samples from up to 50 cows.

For veterinary use only.

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 cattletype Milk Prep Kit is tested against predetermined specifications to ensure consistent product quality.

Storage

The components of the cattletype Milk Prep Kit should be stored at 2-8°C and are stable until the expiration date stated on the label. The spin columns can be stored at room temperature (18-25°C). In order to avoid evaporation, make sure to close reagent bottles (especially the Matrix) tight, when using the reagent kit more than once.

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.

Principle

After separation of the milk casein, IgG-antibodies from milk whey are bound to the special Matrix of the cattletype Milk Prep Kit. Unbound milk components are removed by centrifugation and washing steps. Concentrated antibodies are obtained from the Matrix by an elution step and can be assayed in ELISA procedures.

Equipment and reagents to be supplied by user

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

- Centrifuge suitable for 2, 15, and 50 ml reaction tubes
- Pipets (adjustable)
- Rotating platform or shaker
- Optional: water-jet vacuum pump

Important notes

General precautions

The user should always pay attention to the following:

- Components of the test kit should not be contaminated or mixed with components from other batches.
- Do not use the components of the test kit past the expiration date.
- For accurate test results, it is essential to use clean glassware and to pipet and rinse carefully and strictly adhere to the incubation times when processing the samples.

Protocol

Important points before starting

- Please read „Important notes“ on page 6 before starting.

Procedure for pooled and bulk milk samples

1. Pour 50 ml pooled or bulk milk sample in an appropriate plastic tube (e.g., 50 ml blue cap tube). Make sure the tube can be closed tightly with a lid.

   Note: Prepare milk pool by combining 1 ml each of up to 50 individual milk samples. Pooled and bulk milk samples should contain 45-50 ml.

2. Add 1 ml of Precipitation Reagent (green solution). Close the tube tightly and mix contents by inverting the tube upside down 10 times.

   Note: Turn the tube for equal distribution of Precipitation Reagent in the sample. Successful precipitation is indicated by coagulation of milk casein.

3. Centrifuge at 4°C for 15 min at 3800 x *g*.

   Note: Milk casein should be condensed, forming a pellet at the bottom of the sampling device.

   Important: In case there is a cream layer on the milk whey after centrifugation it should be removed.

4. Decant supernatant in a new 50 ml device, add 2 ml of Neutralization Buffer (NB, red solution) and mix by gentle swirling.

   Note: Discard casein pellet. After adding 2 ml NB, the sample color should change from yellow to red. If there is no color change, add NB in 300 µl steps and mix the sample until the color changes to red.

5. Add 800 µl of Matrix-Suspension and incubate sample device for 2 h at room temperature (18-25°C). Gently agitate device during incubation e.g., using a rotating platform.

   Note: The Matrix tends to sediment; therefore it should be rotated during incubation.

6. Centrifuge sample at 4°C for 5 min at 3000 x *g*. Carefully remove and discard supernatant. Allow 3-5 mm supernatant to remain on the matrix pellet.

   Note: In case the Matrix is not condensed to a pellet please extend centrifugation time. For removal of the supernatant we recommend a water-jet vacuum pump.

7. Resuspend the matrix pellet in remaining buffer and add to the Spin Filter.

   Note: Indicate sample identification by writing it on the Spin Filter.

8. Centrifuge tubes for about 3 s and discard buffer from the Collection Tube.

   Note: In case not all the Matrix can be transferred to the Spin Filter at once, the remaining Matrix should be transferred after centrifugation and Step 8 repeated.

9. Rinse Matrix 2 times with 500 µl double-distilled water. Centrifuge as described in Step 8 and discard buffer from the Collection Tube.

   Note: in case not all the Matrix could be transferred during step 7, use Washing Step 1 to collect the remaining Matrix and transfer it to the Spin Filter.

   Note: In case there is a remaining liquid layer on the Matrix, the centrifugation step should be repeated.

10. Add 10 µl Neutralisation Buffer into an empty 1.5 or 2 ml reaction tube and place the Spin Filter from the Collection Tube above.

    Note: Indicate sample identification by writing it on the reaction tube.

11. Add 200 µl Elution Buffer (yellow solution) to the Matrix in the Spin Filter. Incubate for 1-5 min and centrifuge for about 3 s.

    Important: Pay attention that all the Matrix is covered by Elution Buffer.

    Note: Colour change from yellow to pink indicates proper neutralisation of the eluted sample.

12. Eluted sample concentrate can be processed neat as sample specimen in ELISA.

    Note: Depending on the assay, the amount of eluted sample used as specimen may vary and a dilution step might be necessary.

Procedure for individual milk samples

1. Pour 5 ml milk sample in an appropriate plastic tube (e.g., 15 ml blue cap tube). Make sure the tube can be closed tightly with a lid.
2. Add 100 µl of Precipitation Reagent (green solution). Close the tube tightly and mix contents by inverting the tube upside down 10 times.

   Note: Turn the tube for equal distribution of Precipitation Reagent in the sample. Successful precipitation is indicated by coagulation of milk casein.
3. Centrifuge at 4°C for 15 min at 3800 x *g*.

   Note: Milk casein should be condensed, forming a pellet at the bottom of the sampling device.

   Important: In case there is a cream layer on the milk whey after centrifugation it should be removed.
4. Decant supernatant in a new 15 ml device, add 200 µl of Neutralization Buffer (NB, red solution) and mix by gentle swirling.

   Note: Discard casein pellet. After adding 200 µl NB, the sample color should change from yellow to red. If there is no color change, add NB in 50 µl steps and mix the sample until the color changes to red.
5. Add 200 µl of Matrix-Suspension and incubate sample device for 2 h at room temperature (18-25°C). Gently agitate device during incubation e.g., using a rotating platform.

   Note: The Matrix tends to sediment; therefore it should be rotated during incubation.
6. Centrifuge sample at 4°C for 5 min at 3000 x *g*. Carefully remove and discard supernatant. Allow 3-5 mm supernatant to remain on the matrix pellet.

Note: In case the Matrix is not condensed to a pellet please extend centrifugation time. For removal of the supernatant we recommend a water-jet vacuum pump.

7. Resuspend the matrix pellet in remaining buffer and add to the Spin Filter.

   Note: Indicate sample identification by writing it on the Spin Filter.

8. Centrifuge tubes for about 3 s and discard buffer from the Collection Tube.

   Note: In case not all the Matrix can be transferred to the Spin Filter at once, the remaining Matrix should be transferred after centrifugation and Step 8 repeated.

9. Rinse Matrix 2 times with 500 µl double-distilled water. Centrifuge as described in Step 8 and discard buffer from the Collection Tube.

   Note: in case not all the Matrix could be transferred during step 7, use Washing Step 1 to collect the remaining Matrix and transfer it to the Spin Filter.

   Note: In case there is a remaining liquid layer on the Matrix, the centrifugation step should be repeated.

10. Add 10 µl Neutralisation Buffer into an empty 1.5 or 2 ml reaction tube and place the Spin Filter from the Collection Tube above.

    Note: Indicate sample identification by writing it on the reaction tube.

11. Add 200 µl Elution Buffer (yellow solution) to the Matrix in the Spin Filter. Incubate for 1-5 min at room temperature and centrifuge for about 3 s.

    Note: Colour change from yellow to pink indicates proper neutralisation of the eluted sample.

12. Eluted sample concentrate can be processed neat as sample specimen in ELISA.

Note: Depending on the assay, the amount of eluted sample used as specimen may vary and a dilution step might be necessary.

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, cador, 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.

Notes

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Change index

Handbook	Version	Change
HB-1658-002	July 2018	INDICAL design

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