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cattletype® Milk Prep Kit Handbook

Σ 50 (catalog no. 271906)

For concentration and purification of antibodies from milk samples

REF 271906

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Contents

Kit Contents

cattletype Milk Prep Kit	50
Catalog no.	**271906**
Precipitation Reagent, ready-to-use	1 x 60 ml
Neutralization Buffer, ready-to-use	2 x 60 ml
Matrix, ready-to-use	2 x 20 ml
Elution Buffer, ready-to-use	1 x 20 ml
Spin Filters	50
Collection Tubes	50

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.

Storage

The *cattletype* Milk Prep Kit reagents should be stored at 2–8°C. Collection Tubes and Spin Filters can be stored at room temperature. In order to avoid evaporation, make sure to close reagent bottles (especially the Matrix) tight, when using the

reagent kit more than once. When stored correctly *cattletype* Milk Prep Kit is stable until the indicated expiration date.

Symbols

	Contains reagents for <N> preparations
	Legal manufacturer
	Lot number
	Use by date
	Temperature limitations for storage
	Handbook
	Catalog number
	Material number

Test 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.

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 online in convenient and compact PDF format at www.qiagen.com/safety where you can find, view, and print the SDS for each QIAGEN kit and kit component.

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

24-hour emergency information
Chemical emergency or accident assistance is available 24 hours a day from:
CHEMTREC
USA & Canada ▪ Tel: 1-800-424-9300
Outside USA & Canada ▪ Tel: +1-703-527-3887 (collect calls accepted)

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of *cattletype* Milk Prep Kit is tested against predetermined specifications to ensure consistent product quality.

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
- Water-jet vacuum pump (optional)

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 expiration date.
- The use of clean glass devices, careful pipetting and rinsing during the test, and strict adherence to the indicated incubation times is essential for precise test results.

Protocol 1: For pooled and bulk milk samples

Procedure

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.**

   Prepare milk pool by combining 1 ml each of up to 50 individual milk samples. 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.**

   Turn the tube for equal distribution of the 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*.**

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

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

   Important: In case there is a cream layer on the milk whey it should be removed. 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 200 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.**

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.**
   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. **Put a Spin Filter into a Collection Tube. Re-suspend the matrix pellet in remaining buffer and add to the Spin Filter.**
   Indicate sample identification by writing it on the Spin Filter.
8. **Centrifuge tubes for about 3 s and discard buffer from the Collection Tube.**
   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.**
   In case there is a remaining liquid layer on the Matrix, the centrifugation step should be repeated.
10. **Add 10 µl Neutralisation Buffer into reaction tubes and transfer Spin Filter from Collection Tube to the reaction tube.**
    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 minutes and centrifuge for about 3 s.**
    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.**
    Depending on the assay, the amount of eluted sample used as specimen may vary and a dilution step might be necessary.

Protocol 2: For individual milk samples

Procedure

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 tight with a lid.**
2. **Add 100 µl of Precipitation Reagent (green solution). Close the tube tightly and mix content by turning the tube upside down 10 times.**

   Turn the tube for equal distribution of the 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*.**

   Milk casein should be condensed, forming a pellet at the bottom of the sampling device.
4. **Decant supernatant in a new 15 ml device and add 200 µl of Neutralization Buffer (NB; red solution) and mix by gentle swirling.**

   Important: In case there is a cream layer on the milk whey it should be removed. Discard casein pellet. After adding 200 µl NB, sample color should change from yellow to red. If there is no color change, add Neutralization Buffer in 200 µl steps and mix sample until 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.**

   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.**
   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. **Put a Spin Filter into a Collection Tube. Re-suspend the matrix pellet in remaining buffer and add to the Spin Filter.**
   Indicate sample identification by writing it on the Spin Filter.
8. **Centrifuge tubes for about 3 s and discard buffer from the Collection Tube.**
   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 Collection Tube.**
   In case there is a remaining liquid layer on the Matrix, the centrifugation step 8 should be repeated.
10. **Add 10 µl Neutralisation Buffer into reaction tubes and transfer Spin Filter from Collection Tube to the reaction tube.**
    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 minutes and centrifuge for about 3 s.**
    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.**
    Depending on the assay, the amount of eluted sample used as specimen may vary and a dilution step might be necessary.

Ordering Information

Product	Contents	Cat. no.
cattletype Milk Prep Kit (50)	Precipitation Reagent, Neutralization Buffer, Matrix, Elution Buffer, Spin Filters, Collection Tubes	271906
Related product		
cattletype BHV1 gB Ab (5)	For 480 reactions: 5 Test Plates (strips), Wash Solution, Sample Diluent, Positive Control, Negative Control, Conjugate, TMB Substrate, Stop Solution	270043
cattletype BHV1 gB Ab (20)	For 1920 reactions: 20 Test Plates, Wash Solution, Sample Diluent, Positive Control, Negative Control, Conjugate, TMB Substrate, Stop Solution	270045

QIAGEN offer a range of ELISA kits and real-time PCR and real-time RT-PCR kits for the detection of animal pathogens. Visit www.qiagen.com/Animal-and-Veterinary-Testing for more information about *cattletype*.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.
Notes

Limited License Agreement for *cattletype* Milk Prep Kit.

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