

PROTEIN EXTRACT OF Besnoitia besnoiti

PRODUCT DESCRIPTION

Protein extract is obtained from *Besnoitia besnoiti* tachyzoites growth in cell cultures of the MARC-145 cell line. Protein extract is produced from tachyzoites purified by gel filtration that removes essentially all MARC-145 cell proteins and next, tachyzoites arelyophilized.

The protein extract has been optimized and is batch-to-batch tested to be used as antigen in ELISA for serological diagnosis of the besnoitiosis in cattle and other ruminants¹.

REAGENTS

The protein extract is provided as a powder.

PROCEDURE

Resuspend the vial in the desired buffer for coating ELISA plates. Initially, it is recommended to add a small volume of approximately 2-4 ml and mix well. Subsequently, complete up to the necessary volume for coating ten 96-well plates. Finally, homogenize the content vigorously.

PRODUCT PROFILE

We include specific information about the optical density values from negative and positive reference bovine sera in the quality product report. Basically microtiter plates coated with protein extract in are 0.05 carbonate/bicarbonate buffer, pH 9.6. An anti-lgG1/lgG2 Μ bovine antibody conjugated with peroxidase and ABTS (2,2'-Azinobis [3ethylbenzothiazoline-6-sulfonic acid]-diammonium salt) as colorimetric substrate at 25 °C are used.



Negative and positive reference bovine sera from experimentally and naturally infected animals with *B. besnoiti* are employed and the ratio obtained between optical density achieved by positive and negative control sera is \geq 12.

STORAGE

For extended storage, the powder should be frozen at – 80 °C. We recommend the use of the protein extract for a period not longer than a year to avoid protein degradation.

References

 García-Lunar P, Ortega-Mora L. M, Schares G, Diezma-Díaz C, Álvarez-García G. A new lyophilized tachyzoite based ELISA to diagnose Besnoitia spp. infection in bovids and wild ruminants improves specificity.



Besnoitia besnoiti FORMALDEHYDE-FIXED TACHYZOITES

PRODUCT DESCRIPTION

Besnoitia besnoiti tachyzoites are growth in cell cultures of the MARC-145 cell line. Tachyzoites are purified by gel filtration that removes essentially all MARC-145 cell debris and fixed in buffered 0.2% formaldehyde.

Formaldehyde-fixed *B. besnoiti* tachyzoites production has been optimized and is batch-to-batch tested to be used as antigen in IFAT for serological diagnosis.

REAGENTS

Fixed B. besnoiti tachyzoites are provided as a suspension in phosphate buffered saline, pH 7.4, containing 0.2 % formaldehyde*. Besnoitia besnoiti isolate is the Sp1.

PRODUCT PROFILE

Each vial contains 10^7 formaldehyde-fixed tachyzoites. We recommend homogenizing the tachyzoite suspension by passing through 25G needle prior to each use. The recommended number of tachyzoites per 4 mm-diameter well is 10^5 tachyzoites (10 µl of tachyzoite suspension/ well) for IFAT analysis.

STORAGE

Keep the vials refrigerated at a temperature between 2 and 8 ° C up to one year. Do not freeze.

* This product contains formaldehyde. Use appropriate equipment and avoid inhalation and contact with skin and eyes. Consult formaldehyde hazard and precautionary statements before use.



Besnoitia besnoiti TACHYZOITES

PRODUCT DESCRIPTION

Besnoitia besnoiti tachyzoites are obtained from cell cultures of the MARC-145 cell line. Tachyzoites are purified by gel filtration that removes essentially all MARC-145 cell debris. Tachyzoites are washed with PBS containing 2 mM of protease inhibitor PMSF* as a preservative and pelleted by centrifugation.

REAGENTS

Besnoitia besnoiti tachyzoites are provided as a pellet. Besnoitia besnoiti isolate is Bb-Sp1.

PRODUCT PROFILE

Besnoitia besnoiti tachyzoites can be used for the extraction of nucleic acids or proteins from the parasite. Tachyzoites can be also used as *B. besnoiti* antigen for Western blotting by resuspending the tachyzoite pellet in lysis buffer or directly acrylamide gel's SDS-loading buffer and heating > 90°C for 2 minutes. We include in the product package label specific information about the amount of tachyzoites per pellet.

STORAGE

The pellet should be frozen at – 80 $^{\circ}$ C up to one year. Repeated freezing and thawing is not recommended.

* This product contains PMSF. Use appropriate equipment and avoid inhalation Consult PMSF hazard and precautionary statements before use.



Besnoitia besnoiti DNA

PRODUCT DESCRIPTION

Genomic DNA from *Besnoitia besnoiti* is obtained from purified tachyzoites growth in cell cultures of the MARC-145 cell line. DNA extraction is carried out by conventional methods removing RNA by RNase treatment.

REAGENTS

Besnoitia besnoiti DNA is provided in a double distilled and deionized water solution (molecular biology quality).

PRODUCT PROFILE

Besnoitia besnoiti DNA can be used as template and positive control for B. besnoiti PCR and quantitative PCR. We include specific information about the concentration and purity of DNA determined by spectrophotometry at 280/260 nm in the product package label. The equivalence of DNA concentration present in a 1 microlitter of DNA solution with the number of tachyzoites is also given.

STORAGE

DNA solution should be frozen at -20 °C up to one year. For extended storage, the solution should be frozen at – 80 °C (specially for quantitative PCR use). Repeated freezing and thawing is not recommended. Prepare aliquots of DNA solution previous to storage.