

Dolfinin Code:
Product Name:

Dolfinin Chlamydia CFT bulk
Bulk antigen for diagnosing Chlamydia by CFT

- Specifications:** A white or slightly yellow suspension of highly purified *Chlamydophila psittaci* cells in phosphate – buffered saline with thiomersal as a preservative. A sediment is formed after a longer period of storage. The sediment can easily be resuspended by shaking. The antigen has a titer of 24.
- Indication:** The antigen is used for the detection of antibodies against *Chlamydia* in the blood serum samples of humans and animals by the complement fixation (CF) test. It is very useful for the serological screening and surveillance.
- Testing:** Shake the antigen well before use and prepare its working dilution of 1 + 11 using the buffered solution. A working dilution 1+23 can also be used. It is recommended to perform a control test close to the antigen expiration date to prove whether both antigen dilutions give similar results.
- Preparation of the buffered saline solution for both the macromethod and micromethod:**
Dissolve 85.0 g NaCl and 3.75 g 5,5-sodium diethyl barbiturate in 500 ml of redistilled water. Then dissolve 5.75 g of 5,5-diethyl barbituric acid, MgCl₂·6H₂O (1.68 g), and CaCl₂·2H₂O (0.37 g) in 500 ml of hot redistilled water. Mix both solutions and let the resulting solution cool to room temperature, and finally fill it up to 2000 ml.
Dilute the solutions with redistilled water in a 1 + 4 proportion before use.
Titrate the amboceptor according to the common laboratory procedures.
- Complement titration:** Complement titration is carried out in the presence of the antigen used for the titration itself in its working dilution of 1 + 11 (with buffered saline). Use a method given for the respective complement.
- Serum examination:**
- Macromethod:** Serum titration is accomplished by the warm method using the antigen with its working dilution of 1 + 11, 2 MHD of complement, and 2 MHD of amboceptor. Inactivate the serum at 56 °C for 30 minutes before use. Dilute the serum in buffered saline solution in a series of 1 : 4, 1 : 8, 1 : 16, 1 : 32, etc. Add 0.1 ml of the antigen (at the working dilution of 1 + 11) and 0.1 ml of complement (2 MHD) to each 0.1 ml of serum dilution. Incubate tubes at 37 °C in a water bath for 90 minutes, then add 0.2 ml of the hemolytic system. Incubate again at 37 °C in a water bath for 30 minutes.
- Micromethod:** It is performed in the U shaped microtiter plates. Serum titration is accomplished by the warm method using the antigen with its working dilution of 1 + 11, 2 MHD of complement, and 2 MHD of amboceptor. Inactivate the serum at 56 °C for 30 minutes before use. Dilute the serum in buffered saline solution in a series of 1 : 4, 1 : 8, 1 : 16, 1 : 32, etc. Add 25 µl of the antigen (at the working dilution of 1 + 11) and 25 µl of complement (2 MHD) to each 25 µl of serum dilution. Incubate plates at 37 °C for 30 minutes, then add 50 µl of the hemolytic system. Incubate again at 37 °C for 30 minutes. After incubation, the plates are centrifuged and read or the plates are left at 4 °C for 12-24 h and then read.
Standard negative and positive sera are used as controls.
- Test evaluation:**
- Macromethod:** ++++ = full haemolysis impediment
+++ , ++ , + = haemolysis impediment in a gradually decreasing intensity
- = blood cells fully dissolved
Titer of the serum examined gives its highest dilution in which at least +++ reaction can be observed.
- Micromethod:** a positive result = full haemolysis impediment; on the bottom of the well a red “button” is formed
a negative result = blood cells fully dissolved; a clear well
- Storage:** Store in a dark and dry place at the temperature of 2 – 8 °C.
- Expiration date:** One year from the date of manufacture.

Complement* titration

Complement titration:

Complement titration is carried out in the presence of antigen used for the titration itself in its working dilution of 1 + 11 for *C. psittaci* antigen.

Carry out the titration according to the following table:

Tubes No:	1	2	3	4	5	6	7	8	9	10
Complement 4% 1 : 25 (ml)	0.20	0.16	0.14	0.12	0.10	0.08	0.07	0.06	0.04	0.02
Buffered saline (ml)	0.10	0.14	0.16	0.18	0.20	0.22	0.23	0.24	0.26	0.28
Antigen (ml)	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10

Incubation at 37°C for 30 minutes

Hemolytic system = ambo- ceptor 2 MHD + the same portion of 2% blood cells (ml)	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
---	------	------	------	------	------	------	------	------	------	------

Incubation at 37°C for 30 minutes

Complement dilution (1MHD)	12.5	15.625	17.86	20.83	25	31.25	35.70	41.66	62.5	125
(2MHD)	6.25	7.812	8.93	10.42	12.5	15.625	17.85	20.83	31.25	62.5

*Complement – supplied by Dolfin, spol. s.r.o.