



## Anti-Toxoplasma gondii ELISA Cat (IgG) Avidity determination



- **Alternative principle for the detection of fresh infections**
- **No false negative results due to a lack of IgM-antibodies**
- **Efficient automation solutions available**



### Technical data

<b>Antigen</b>	Detergent extract of purified <i>Toxoplasma gondii</i> organisms
<b>Avidity determination</b>	Calculation of a relative avidity index (RAI) from the extinction of a sample which is analysed twice, with and without urea treatment
<b>Result interpretation</b>	EUROIMMUN recommends interpreting results as follows: RAI < 40%: Indication of low-avidity IgG antibodies RAI 40–60%: Borderline range RAI > 60%: Indication of high-avidity IgG antibodies
<b>Sample dilution</b>	Feline serum or plasma, 1:101 in sample buffer
<b>Reagents</b>	Ready for use, with the exception of the wash buffer (10x); colour-coded solutions
<b>Test procedure</b>	30 min (37 °C) / 10 min (room temperature) / 30 min (37 °C) / 15 min (room temperature), fully automatable
<b>Measurement</b>	450 nm, reference wavelength between 620 nm and 650 nm
<b>Test kit format</b>	96 break-off wells; kit includes all necessary reagents
<b>Order no.</b>	<b>EI 2410-9601-1 GF</b>



### Clinical significance

The sporozoan *Toxoplasma gondii* is the causative agent of the worldwide distributed zoonosis toxoplasmosis. The only final hosts are the domestic cat and other felidae, in the intestine of which oocysts develop in a sexual development stage. During asexual development, which can also take place in other warm-blooded animals and in birds, the *Toxoplasma* parasites develop in brain, muscle, liver, spleen and in other organs, where they become encapsulated. Humans are generally infected perorally by ingestion of water or food contaminated with oocysts (through the faeces of infected cats) or from meat products (the raw flesh of infected animals contains cysts with viable trophozoites).

Cats become infected primarily by ingestion of infected rodents or other raw meat, less frequently through ingested oocysts or by intrauterine infection. It could be shown that cerebral toxoplasma cysts cause behavioural changes in mice and rats, which increase the rodent's probability of getting caught by a cat. *Toxoplasma* infections in cats proceed asymptotically in most cases. Especially congenitally infected cats, however, often develop severe clinical symptoms, from which they die. Proliferation of the parasite in the intestine of the host can lead to diarrhoea. The infection of extraintestinal tissue frequently affects the lungs, liver, CNS, pancreas or eyes. In this case, the cats present with lethargy, anorexia, fever, icterus, dyspnoea, ataxia or uveitis.

Alongside the clinical relevance for cats, toxoplasmosis is an important zoonosis. After primary infection or reactivation of a latent infection (e.g. by immunosuppression or reinfection), infected cats excrete oocysts with their faeces for 1 to 3 weeks. These become infectious after 2 to 4 days in the environment and can perorally infect humans or warm-blooded animals. Postnatal infection is often symptom-free. However, in immunosuppressed individuals the parasites can cause severe infections, such as encephalitides in AIDS patients, even after reactivation. In pregnant women and warm-blooded animals *Toxoplasma* can be transmitted via the placenta to the foetus. Intrauterine infection can result in abortion, malformation and other damage to the newborn, depending on the time and dose of infection and the immune status of mother and foetus.



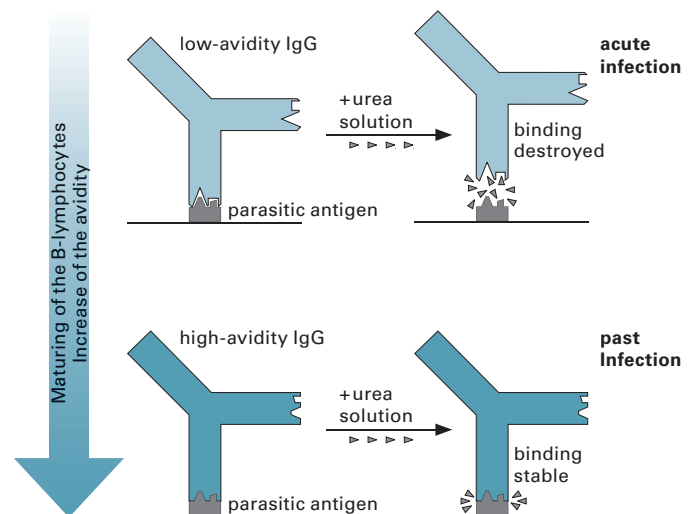
## Diagnostic application

To date, the most common means to differentiate between fresh and latent infections has been the investigation of specific IgM class antibodies, which, in general, only appear during the initial phase but whose determination is often unreliable and problematic. Interfering factors are the persistence of the IgM response, too weak or delayed IgM production, and unspecific IgM production through polyclonal B cell stimulation. Moreover, it should be taken into account that around 20% of infected cats do not develop detectable levels of IgM antibodies against *Toxoplasma gondii* and that additionally cases of reactivated toxoplasmosis have been described that did not exhibit specific IgM. Further, persisting IgM antibodies are present after infection with *Toxoplasma gondii*. An alternative to their detection is the determination of avidity of specific IgG antibodies. The first reaction of the immune system following an infection is the formation of low-avidity antibodies. With ongoing progression of the disease, the secreted IgG becomes more and more adapted to the antigen– the avidity increases. If high-avidity IgG is detectable in the serum, it can be assumed that the infection has been present for a longer time.



## Principle of the test: Determination of low-avidity IgG antibodies

The first reaction of the immune system following an infection is the formation of low-avidity antibodies. As the infection proceeds, antigen-adapted IgG is increasingly formed, and avidity grows. If high-avidity IgG is detected in the serum, it can be assumed that the infection is in a late stage. In order to identify low-avidity antibodies in a patient sample, the sample is tested in two preparations in the ELISA. After sample incubation, one of the preparations is treated with urea. Low-avidity antibodies detach from the antigens while the second sample remains untreated. Subsequently, the samples are incubated with peroxidase-labelled anti-cat IgG. The presence of low-avidity antibodies in a patient's serum has been proved if the ELISA extinction is significantly reduced by urea treatment. For an objective interpretation the relative avidity index (RAI) can be calculated out of the measured values with and without urea incubation and expressed as a percentage.



## Sensitivity and specificity

To determine sensitivity and specificity, precharacterised feline samples were analysed with the Anti-*Toxoplasma gondii* ELISA Cat (IgG) Avidity determination. In samples from cats that were experimentally infected with *Toxoplasma gondii*, the same samples were characterised as freshly infected as with a commercially available IIFT approved by the Friedrich Loeffler Institute (FLI).

Sample	Days after inoculation	EUROIMMUN Anti- <i>Toxoplasma gondii</i> ELISA Cat		EUROIMMUN Anti- <i>Toxoplasma gondii</i> IIFT Cat (FLI-B 567) <sup>1</sup>	
		IgG	IgG avidity	IgG	IgM
R09-49-1	8	negative	not evaluable <sup>2</sup>	negative	negative
R15-15	10	borderline	low-avidity	positive	positive
R07-06	14	borderline	low-avidity	positive	positive
R06-13	26	positive	low-avidity	positive	positive

<sup>1</sup> Approved in accordance with § 17 c TierSG; <sup>2</sup> (Approval no.: FLI-B 567); <sup>2</sup> OD < 0.140

The analysis of a total of 306 randomly selected feline samples yielded an amount of 77 samples with IgG antibodies against *Toxoplasma gondii*. Out of the 306 investigated feline samples, 229 were IgG-negative and therefore not evaluable with respect to avidity. 5 out of the 77 evaluable samples had a low-avidity result, which indicates an acute infection. 18 samples showed a borderline result, which indicates a relatively fresh infection in the convalescent phase. 54 samples showed a high avidity, which indicates a past infection.

n = 306	EUROIMMUN Anti- <i>Toxoplasma gondii</i> ELISA Cat (IgG) Avidity determination
negative for IgG	229
low-avidity IgG	5
borderline range	18
high-avidity IgG	54