# Equine IL-10 ELISA

**Cat.n° 38670109**

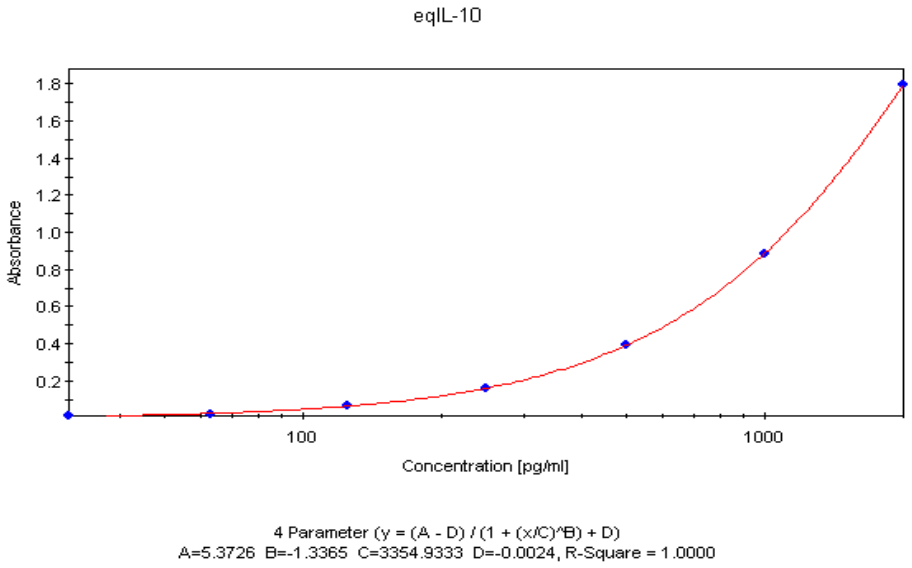
This ImmunoTools ELISA for **equine Interleukin-10 (IL-10)** contains appropriate reagents sufficient for processing of 5 microplates (5 x 96 wells; 100 µl/well)

**Typical standard curve range**: 31– 2000 pg/ml

**Detection limit under optimal conditions**: 27 pg/ml

| Content | Working dilution | Storage |
|---|---|---|
| 1 x vial 500 µl liquid anti-eqIL-10 Capture-Antibody (red cap) | 1:100 | -20°C |
| 1 x vial 500 µl liquid anti-eqIL-10 Detection-Antibody (yellow cap) | 1:100 | -20°C |
| 1 x vial 50 ng lyophilized req IL-10 Standard (white cap) | customer specific | -20°C |
| 1 x vial 50 µl Poly-HRP-Streptavidin (blue cap) | 1:1000 | -20°C |

**Additional material required:**
96well-Microplates
Wash-Buffer (e.g. PBS + 0.05% Tween20)
Coating-Buffer (e.g. PBS)
Blocking-Buffer / Reagent-Diluent (e.g. PBS + 2% BSA + 0.05% Tween20)
Stop-Solution (e.g. 2 M $H_2SO_4$)
TMB-Solution




**Note:** All steps of incubation except HRP-Streptavidin and TMB substrate could be carried out overnight. Do not use solutions containing sodium azide, nor add sodium azide to the supplied reagents. Sodium azide inactivates the peroxidase.

**Storage:** Protect from light! Storage conditions are shown in the table above.
Reconstituted reagents should be stored at -20° C. Please prevent repeated freeze-thaw cycles. The performance of the unopened reagents is guaranteed until the expiration date shown on the label.

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Gladiolenweg 2; 26169 Friesoythe; Germany
phone:+49-(0)4491-400997, fax:+49-(0)4491-400998, info@immunotools.com
www.immunotools.com

Spin down all vials before use!

| Step | Incubation | Procedure |
|---|---|---|
| **Coating Capture-antibody** | ≥ OVERNIGHT at room temperature | Dilute capture-antibody 1:100 in COATING-BUFFER (100 µl capture-antibody in 10 ml COATING-BUFFER). Subsequently transfer 100 µl of this working-solution to each well and incubate. |
| Remove capture-antibody completely by inverting the microplate and blotting it ***vigorously*** against clean paper towels. | | |
| **Blocking** | ≥ 1 Hour at room temperature | Add 300 µl BLOCKING-BUFFER to each well and incubate. |
| Remove BLOCKING-BUFFER completely by inverting the microplate and blotting it ***vigorously*** against clean paper towels. | | |
| **Standard & Sample** | ≥ 2 Hours at room temperature | Dilute standard & samples in REAGENT-DILUENT and transfer 100 µl in the respective wells in duplicates. Standard: Make serial dilutions in REAGENT-DILUENT and begin with a high standard and end with blanks. The standard vial of this set contains **50 ng lyophilized standard**. Reconstitute this in exactly 1 ml REAGENT-DILUENT (stock solution = 50 ng/ml) and choose a sufficient high standard working solution for your assay |
| Wash 5x ***vigorously*** with WASHING-BUFFER and remove resting buffer completely by inverting the microplate and blotting it ***vigorously*** against clean paper towels. | | |
| **Detection-antibody** | ≥ 2 Hours at room temperature | Dilute detection-antibody 1:100 in REAGENT-DILUENT (100 µl detection-antibody in 10 ml REAGENT-DILUENT). Subsequently transfer 100 µl of this working-solution to each well and incubate. |
| Wash 5x ***vigorously*** with WASHING-BUFFER and remove resting buffer completely by inverting the microplate and blotting it ***vigorously*** against clean paper towels. | | |
| **Poly-HRP-Streptavidin** | 30 Min at room temperature | Dilute Poly-HRP-Streptavidin 1:1000 in REAGENT-DILUENT (10 µl in 10 ml REAGENT-DILUENT). Subsequently transfer 100 µl of this working-solution to each well and incubate. |
| Wash 5x ***vigorously*** with WASHING-BUFFER and remove resting buffer completely by inverting the microplate and blotting it ***vigorously*** against clean paper towels. | | |
| **Substrate solution** | Up to 60 Min* at room temperature **in the dark** | Optionally warm the solution to room temperature before use. Add 100 µl of the SUBSTRATE-SOLUTION to each well and incubate. Control the development of the colour reaction continuously and stop at an appropriate time point. |
| **Stop solution** | - | When the enzymatic colour reaction is sufficiently proceeded stop the reaction by adding of 50 µl stop solution. Read the microplate at the substrate-depending wavelength. (e.g. **450** nm for TMB substrate) (if wavelength correction is available, set to 540 nm, 570 nm or 630 nm as reference) |

*The speed of enzymatic colour development is influenced by many customer-specific factors. Therefore the incubation time is variable und specific for each test system.

**Warning:** TMB-Solution is harmful if swallowed (R22). Keep out of reach of children (S2). Keep away from food, drink and animal feeding stuff (S13). Wear suitable protective clothing (S36). If swallowed, seek medical advice immediately and show this container or label (S46). Contact with acids liberates very toxic gas (R32). Azide compounds should be flushed with large volumes of water during disposal to avoid deposits in lead or copper plumbing where explosive conditions can develop.

This material is offered for **research only.** Not for use in diagnostic or therapeutic procedures.Not for use in human. For in vitro use only. ImmunoTools will not be held responsible for patent infringement or other violations that may occur with the use of our products.

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