# Swine IL-10 ELISA Kit

**Catalog Number** KSC0101 (96 tests), KSC0102 (2 × 96 tests)

**Pub. No.** MAN0014763 **Rev.** 4.0 (31)

**CAUTION!** This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state, and local regulations for disposal.

**Note:** For safety and biohazard guidelines, see the "Safety" appendix in the *ELISA Technical Guide* (Pub. no. MAN0006706). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

## Product description

The Invitrogen™ Swine IL-10 ELISA Kit is a solid-phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA). This assay is designed to detect and quantify the level of Swine IL-10 in swine serum, swine plasma, buffered solution, or cell culture medium. The assay will recognize both natural and recombinant Swine IL-10.

Interleukin-10 (Hu IL-10) is a lymphokine produced by T helper lymphocytes, monocytes, macrophages and B-lymphocytes. IL-10 was first characterised as a cytokine synthesis inhibitory factor (CSIF) able to inhibit cytokine synthesis by Th1 clones activated in the presence of antigen presenting cells. However, in the absence of monocytes, IL-10 directly inhibits the growth of T-cells triggered by immobilised anti-CD3 monoclonal antibody. This inhibition of proliferation is a result of IL-2 production by the responding T-cells. In vitro, IL-10 is a very powerful inhibitor of monokines (including TNF-$\alpha$, IL-1, IL-6 and IL-8) produced by LPS-activated monocytes and macrophages. The addition of IL-10 to B lymphocytes results in limited cell proliferation but most importantly in very high immunoglobulin production, a result of the transformation of B-cells into plasma cells. Finally, natural killer (NK) cells appear to be another target for the anti-inflammatory properties of IL-10. Indeed, recent data have shown that IL-10 can inhibit antigen induced IFN-$\gamma$ production by NK-cells by inhibiting not only production but also the stimulatory effects of IL-12 and TNF on IFN-$\gamma$ production.

## Contents and storage

Upon receipt, store the kit at 2°C to 8°C.

| Contents | Cat. no. KSC0101 (96 tests) |
|---|---|
| Sw IL-10 Standard, lyophilized, recombinant Sw IL-10; contains 0.1% sodium azide. Refer to vial label for reconstitution volume | 2 vials |
| Standard Diluent Buffer; contains 0.1% sodium azide | 25 mL |
| Antibody Coated Plate, 96-well plate | 1 plate |
| Sw IL-10 Biotin Conjugate (Biotin-labeled anti-IL-10); contains 0.1% sodium azide | 11 mL |
| Streptavidin-HRP (100X) | 0.15 mL |
| Streptavidin-HRP Diluent; contains 3.3 mM thymol | 25 mL |
| Wash Buffer Concentrate (25X) | 100 mL |
| Stabilized Chromogen, Tetramethylbenzidine (TMB) | 25 mL |
| Stop Solution | 25 mL |
| Plate Covers, adhesive strips | 3 |

## Materials required but not supplied

- Distilled or deionized water
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solutions; beakers, flask and cylinders for preparation of reagents
- Microtiter plate reader with software capable of measurement at or near 450 nm
- Plate washer–automated or manual (squirt bottle, manifold dispenser, or equivalent)

## Before you begin

**IMPORTANT!** Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

- Review the **Procedural guidelines** and **Plate washing directions** in the *ELISA Technical Guide* available at **thermofisher.com**.
- Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.

## Prepare 1X Wash Buffer

1. Dilute 16 mL of Wash Buffer Concentrate (25X) with 384 mL of deionized or distilled water. Label as 1X Wash Buffer.
2. Store the concentrate and 1X Wash Buffer in the refrigerator. Use the diluted buffer within 14 days.

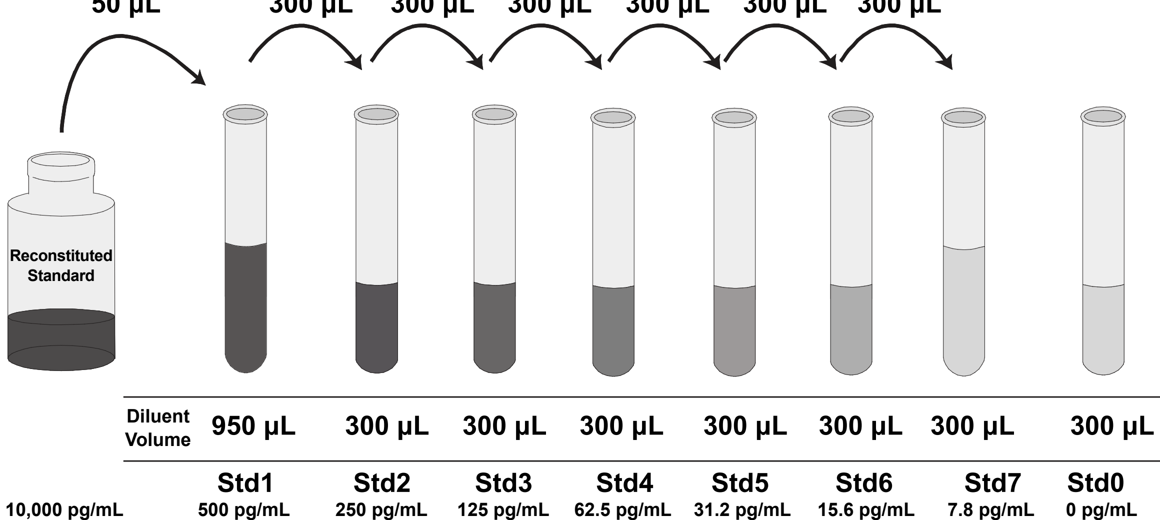
## Sample preparation guidelines

- Refer to the *ELISA Technical Guide* at **thermofisher.com** for detailed sample preparation procedures.
- Collect samples in pyrogen/endotoxin-free tubes.
- Freeze samples after collection if samples will not be tested immediately. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well (do not vortex) prior to analysis.
- Avoid the use of hemolyzed or lipemic sera. If large amounts of particulate matter are present in the sample, centrifuge or filter sample prior to analysis.

## Dilute standards

Note: Use glass or plastic tubes for diluting standards.

1. Reconstitute Swine IL-10 Standard to 10,000 pg/ml with Standard Dilution Buffer. Refer to the standard vial label for instructions. Swirl or mix gently and allow the contents to sit for 10 minutes to ensure complete reconstitution. Label as 10,000 pg/ml Swine IL-10. **Use the standard within 1 hour of reconstitution.**
2. Add 50 µL Reconstituted Standard to one tube containing 950 µL Standard Diluent Buffer and mix. Label as 500 pg/ml Swine IL-10.
3. Add 300 µL Standard Diluent Buffer to each of 7 tubes labeled as follows: 250, 125, 62.5, 31.2, 15.6, 7.8, and 0 pg/ml Swine IL-10.
4. Make serial dilutions of the standard as shown in the following dilution diagram. Mix thoroughly between steps.
5. Remaining reconstituted standard should be discarded or frozen in aliquots at –80°C for further use. Standard can be frozen and thawed one time only without loss of immunoreactivity.




## Prepare 1X Streptavidin-HRP solution

Note: Prepare 1X Streptavidin-HRP within 15 minutes of usage.

To ensure accurate dilution:

1. For each 8-well strip used in the assay, pipet 10 µL Streptavidin-HRP (100X) solution, wipe the pipette tip with clean absorbent paper to remove any excess solution, and dispense the solution into a tube containing 1 mL of Streptavidin-HRP Diluent. Mix thoroughly.
2. Return the unused Streptavidin-HRP (100X) solution to the refrigerator.

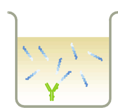
## Perform ELISA (Total assay time: 3 hours)

**IMPORTANT!** Perform a standard curve with each assay.

- Allow all components to reach room temperature before use. Mix all liquid reagents prior to use.
- Determine the number of 8-well strips required for the assay. Insert the strips in the frames for use. Re-bag any unused strips and frames, and store at 2°C to 8°C for future use.

**Capture antibody** **Antigen** **Biotin conjugate** **Streptavidin-HRP**

**1 Bind antigen**



a. Add 100 µL of standards to the appropriate wells. For buffered solutions or cell culture samples, add 100 µL of sample to each well. For controls, plasma and serum samples, add 50 µL of Standard Diluent Buffer followed by 50 µL of sample (see “Pre-dilute samples” on page 2) to the appropriate wells. Leave the wells for chromogen blanks empty.
b. Cover the plate with a plate cover and incubate for 1 hour at room temperature.
c. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

**2 Add Biotin Conjugate**

a. Add 100 µL Swine IL-10 Biotin Conjugate solution into each well except the chromogen blanks.
b. Cover the plate with plate cover and incubate for 1 hour at room temperature.
c. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

**3 Add Streptavidin-HRP**

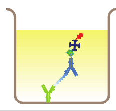
a. Add 100 µL 1X Streptavidin-HRP solution (see page 2) into each well except the chromogen blanks.
b. Cover the plate with a plate cover and incubate for 30 minutes at room temperature.
c. Thoroughly aspirate the solution from the wells and wash wells 4 times with 1X Wash Buffer.

**4 Add Stabilized Chromogen**

a. Add 100 µL Stabilized Chromogen to each well. The substrate solution begins to turn blue.
b. Incubate for 30 minutes at room temperature in the dark.

**Note:** TMB should not touch aluminum foil or other metals.

**5 Add Stop Solution**



Add 100 µL Stop Solution to each well. Tap the side of the plate to mix. The solution in the wells changes from blue to yellow.

## Read the plate and generate the standard curve

1. Read the absorbance at 450 nm. Read the plate within 2 hours after adding the Stop Solution.
2. Use curve-fitting software to generate the standard curve. A 4 parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
3. Read the concentrations for unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.

   **Note:** Dilute samples producing signals greater than the upper limit of the standard curve in Standard Diluent Buffer and reanalyze. Multiply the concentration by the appropriate dilution factor.

## Performance characteristics

### Standard curve example

The following data were obtained for the various standards over the range of 0 to 500 pg/ml Sw IL-10.

| Standard Swine IL-10 (pg/mL) | Optical Density (450 nm) |
|---|---|
| 500 | 2.71 |
| 250 | 1.36 |
| 125 | 0.74 |
| 62.5 | 0.40 |
| 31.2 | 0.22 |
| 15.6 | 0.14 |
| 7.8 | 0.11 |
| 0 | 0.06 |

### Specificity

Buffered solutions of a panel of substances at 10,000 pg/ml were assayed with the Sw IL-10 Kit. The following substances were tested and found to have no cross-reactivity: **Human** IL-1β, IL-2, IL-4, IL-7, IL-8, IL-13, IFN-γ, SCF, TNF-α; **Mouse** IL-1β, IL-2, IL-4, IL-6, IFN-γ, TNF-α; **Rat** IL-1β, IL-2, IL-10, IFN-γ, MCP-1, TNF-α.

### Sensitivity

The analytical sensitivity of Sw IL-10 is < 3.0 pg/ml. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 30 times.

### Cross-reactivity

Significant cross-reactivity was observed to human IL-10.

## Inter-assay precision

Samples were assayed 48 times in multiple assays to determine precision between assays.

| Parameters | Sample 1 | Sample 2 | Sample 3 |
|---|---|---|---|
| Mean (pg/mL) | 58.1 | 180.0 | 324.4 |
| Standard Deviation | 4.2 | 8.8 | 30.5 |
| % Coefficient of Variation | 7.2 | 4.9 | 9.4 |

## Intra-assay precision

Samples of known Sw IL-10 concentration were assayed in replicates of 16 to determine precision within an assay.

| Parameters | Sample 1 | Sample 2 | Sample 3 |
|---|---|---|---|
| Mean (pg/mL) | 59.9 | 182.0 | 311.5 |
| Standard Deviation | 3.8 | 7.3 | 15.7 |
| % Coefficient of Variation | 6.3 | 4.0 | 5.0 |

## Linearity of dilution

Swine serum containing 447 pg/ml of measured Sw IL-10 was serially diluted in Standard Diluent Buffer over the range of the assay. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient of 0.99.

## Recovery

The recovery of Sw IL-10 added to swine serum averaged 103%. The recovery of Sw IL-10 in plasma averaged 105%. The recovery of Sw IL-10 added to tissue culture medium containing 1% fetal bovine serum averaged 87%, while the recovery of Sw IL-10 added to tissue culture medium containing 10% fetal bovine serum averaged 92%. Sera and plasma from Yorkshire and Chester-White pigs have been validated for use in this assay. Other strains of swine have not been tested and consequently their use has not been validated.

| Sample | Average % Recovery |
|---|---|
| Swine serum | 103 |
| Plasma | 105 |
| Tissue culture medium (1% fetal bovine serum) | 87 |
| Tissue culture medium (10% fetal bovine serum) | 92 |

# Limited product warranty

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