# Swine IL-10 ELISA Kit

**Catalog nos.** KSC0101
KSC0102

**Pub. No**. MAN0014763 **Rev** 2.0

## Description

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

## Contents and storage

The components included in the ELISA kit are listed below. Upon receipt, store the kit at 2°C to 8°C.

| Components | Cat. No. KSC0101 96 tests | Cat. No. KSC0102 192 tests |
|---|---|---|
| Sw IL-10 Antibody Coated Wells. 96 well plate. | 1 plate | 2 plates |
| Sw IL-10 Biotin Conjugate (biotin-labeled anti-IL-10 antibody). Contains 0.1% sodium azide. | 11 mL | 2 × 11 mL |
| Sw IL-10 Standard, recombinant Sw IL-10. Lyophilized. Contains 0.1% sodium azide. Refer to vial label for quantity and reconstitution volume. | 2 vials | 4 vials |
| Wash Buffer Concentrate (25X). | 100 mL | 100 mL |
| Standard Diluent Buffer. Contains 0.1% sodium azide. | 25 mL | 2 × 25 mL |
| Streptavidin-HRP (100X). Contains 3.3 mM thymol. | 0.125 mL | 2 × 0.125 mL |
| Streptavidin-HRP Diluent. Contains 3.3 mM thymol. | 25 mL | 25 mL |
| Stabilized Chromogen, Tetramethylbenzidine (TMB). | 25 mL | 25 mL |
| Stop Solution. | 25 mL | 25 mL |
| Adhesive Plate Covers. | 3 | 6 |

**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.

## Materials required but not provided

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

## General guidelines

- Review the **Procedural guidelines** and **Plate washing directions** in the *ELISA Technical Guide* available at **thermofisher.com** for details prior to starting the procedure.
- Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.


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## Prepare 1X Wash Buffer

1. Allow the Wash Buffer Concentrate (25X) to reach room temperature and mix to redissolve any precipitated salts.
2. Dilute 16 mL of Wash Buffer Concentrate (25X) with 384 mL of deionized or distilled water. Label as 1X Wash Buffer.
3. Store the concentrate and 1X Wash Buffer in the refrigerator. Use the diluted buffer within 14 days.

## Sample preparation guidelines

- 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.

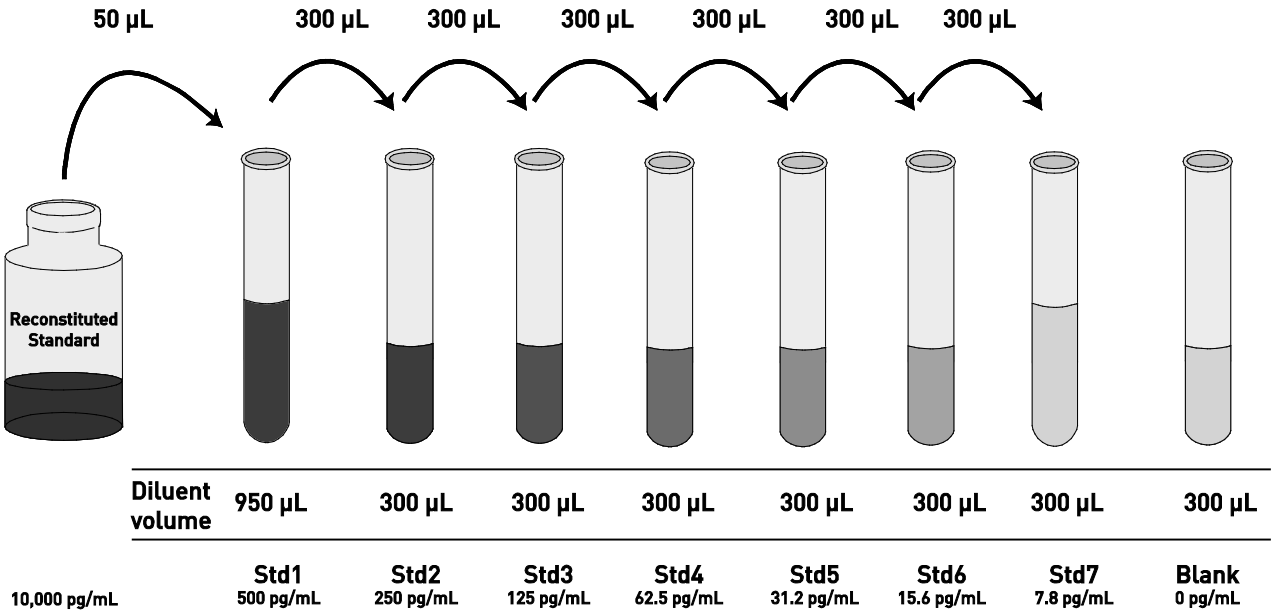
## Dilute samples

- Dilute serum/plasma samples and corresponding medium for cell culture samples > 1000 pg/mL in Standard Diluent Buffer. Because conditions may vary, it is recommended that each investigator determine the optimal dilution to be used for each application.

## Dilute standards

**Note:** Use glass or plastic tubes for diluting standards.

1. Reconstitute Sw IL-10 Standard to 10,000 pg/mL with Standard Diluent 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 Sw IL-10. **Use the standard within 1 hour of reconstitution**.
2. Add 50 µL of reconstituted standard to a tube containing 950 µL of Standard Diluent Buffer and mix. Label as 500 pg/mL Sw 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 Sw IL-10.
4. Make serial dilutions of the standard as shown in the dilution diagram below. Mix thoroughly between steps.
5. Discard any remaining reconstituted standard or freeze in aliquots at -80°C. Return the Standard Diluent Buffer to the refrigerator.




## Prepare 1X Streptavidin-HRP solution

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

The Streptavidin-HRP (100X) is in 50% glycerol, which is viscous. To ensure accurate dilution:

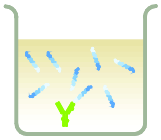
1. Allow Streptavidin-HRP (100X) to reach room temperature and mix gently.
2. For each 8-well strip used in the assay, pipet 10 µL Streptavidin-HRP (100X) solution, wipe the pipette tip with clean, absorbent paper to remove any excess solution, and dispense the solution into a tube containing 1 mL of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

## ELISA procedure

Allow all reagents to reach room temperature before use. Mix all liquid reagents prior to use. **Total assay time is 3 hours.**

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

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 to 8°C for future use.

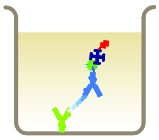


### Bind antigen

1. Add 100 µL of Standard Diluent Buffer to zero wells except the chromogen blanks.
2. 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 to each well followed by 50 µL of sample. Tap the side of the plate to mix.
3. Cover the plate with the plate cover and incubate for **1 hour at room temperature**.
4. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

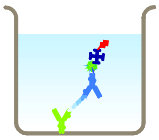
### Add Biotin Conjugate

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



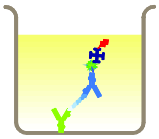
### Add Streptavidin-HRP

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



### Add chromogen

11. Add 100 µL Stabilized Chromogen to each well. The substrate solution will begin to turn blue.
12. Incubate for **30 minutes at room temperature in the dark**.
    **Note:** TMB should not touch aluminum foil or other metals.



### Add stop solution

13. Add 100 µL of Stop Solution to each well. Tap side of the plate gently 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 four 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 serum or plasma samples and controls by 2 to correct for the sample dilution.
4. Dilute samples producing signals greater than that of the highest standard in Standard Diluent Buffer and reanalyze. Multiply the concentration by the appropriate dilution factor.

## Performance characteristics

### Standard curve (example)

Data obtained for the standards over the range of 500 pg/mL Sw IL-10.

| Standard Sw 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. Significant cross-reactivity was observed to human IL-10.

### Sensitivity

The minimum detectable dose of Sw IL-10 is < 3 pg/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 30 times.

## Performance characteristics, continued

### 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 |
| SD | 3.8 | 7.3 | 15.7 |
| %CV | 6.3 | 4.0 | 5.0 |

SD = Standard Deviation; CV = Coefficient of Variation

### 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 |
| SD | 4.2 | 8.8 | 30.5 |
| %CV | 7.2 | 4.9 | 9.4 |

SD = Standard Deviation; CV = Coefficient of Variation

### 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.

### 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.

## Limited product warranty

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## Product label explanation of symbols and warnings

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| REF | Catalog Number | LOT | Batch code | | Temperature limitation | | Use by | | Manufacturer | | Consult instructions for use | | Caution, consult accompanying documents |



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