Version 18-12/25

User's Manual

Chicken Dermatan Sulfate (DS) ELISA Kit

REF **DEIA-NS251014**

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

The Chicken Dermatan Sulfate (DS) ELISA Kit is designed for the quantitative determination of Dermatan Sulfate (DS) concentrations in chicken serum, plasma, cell culture supernatants, and other biological fluids.

For Research Use Only. Not for therapeutic or diagnostic applications!

Principles of Testing

This kit employs a sandwich ELISA technique. The microplate is pre-coated with a monoclonal antibody specific for Chicken Dermatan Sulfate (DS). Samples and biotin-labeled polyclonal antibodies are sequentially added to the wells, allowing DS molecules to form a sandwich complex between the capture and detectionantibodies.

After washing, HRP-conjugated Avidin binds to the biotinylated antibody. Following incubation andwashing steps, the substrate TMB (3,3',5,5'-tetramethylbenzidine) is added. The enzyme-substratereaction produces a blue color that turns yellow upon addition of Stop Solution. The intensity of the color,measured at 450 nm, is directly proportional to the DS concentration in the sample.

Storage

Storage: Store at –20°C. For short-term use, the kit can be kept at 2–8°C for up to 2 weeks.

Shelf Life: 12 months from the production date.

Specimen Collection And Preparation

1. Serum Samples

Collect whole blood and allow it to clot undisturbed at room temperature for 10–20 minutes.

Centrifuge the sample at 2,000–3,000 rpm for 20 minutes to separate the serum.

If any precipitate appears during storage, centrifuge again before testing.

2. Plasma Samples

Collect whole blood in tubes containing an anticoagulant (EDTA or citrate).

Allow the tubes to stand at room temperature for 10–20 minutes, then centrifuge at 2,000–3,000 rpm for20 minutes.

Carefully collect the plasma supernatant. If precipitates form during storage, centrifuge again prior touse.

3. Urine Samples

Collect urine in sterile tubes and centrifuge at 2,000–3,000 rpm for 20 minutes.

Carefully collect the supernatant. If precipitates form during storage, re-centrifuge.

The same procedure applies to cerebrospinal fluid and pleuroperitoneal fluid samples.

4. Cell Samples

For secreted proteins: Collect cell culture supernatant in sterile tubes and centrifuge at 2,000–3,000 rpmfor 20 minutes.

For intracellular proteins: Resuspend cells in PBS (pH 7.2–7.4) at 1×10^6 cells/mL. Lyse the cells byrepeated freeze–thaw cycles, then centrifuge at 2,000–3,000 rpm for 20 minutes.

Collect the supernatant carefully. If precipitates are observed during storage, centrifuge again beforeanalysis.

5. Tissue Samples

Cut and weigh the tissue, then freeze it immediately in liquid nitrogen and store at -80°C until use.

Homogenize the sample in PBS (pH 7.4) at 4°C and centrifuge at 2,000–3,000 rpm for 20 minutes.

Carefully collect and aliquot the supernatant for ELISA testing or future use.

Reagent Preparation

1. Kit Equilibration

Allow the kit and all reagents to reach room temperature (RT) before use.

2. Wash Buffer Preparation

Dilute the concentrated wash buffer (25×) with double-distilled water at a ratio of 1:25. Mix thoroughly to ensure complete dissolution.

Return any unused portion to storage at 2–8°C.

3. Standard Reconstitution

Add 1.0 mL of Standard Diluent to the vial containing the lyophilized standard.

Allow it to dissolve completely for 30 minutes at RT, then mix gently until uniform.

Label this solution as Standard No.0 (10 ng/mL).

Note: Ensure the standard is fully reconstituted and evenly mixed before use.

4. Standard Serial Dilution

Prepare seven labeled tubes (No.1–No.7) as follows:

Add 300 µL of Standard Diluent to each tube.

Transfer 300 µL from Standard No.0 to tube No.1, mix thoroughly.

Sequentially transfer 300 µL from each preceding tube to the next until tube No.6.

Tube No.7 contains only Standard Diluent (blank control).

Recommended standard concentrations for the curve:

Tube	Concentration (ng/mL)	Preparation
No.0	10 ng/mL	Lyophilized Standard + 1.0 mL Standard Diluent
No.1	5 ng/mL	300 µL No.0 + 300 µL Diluent
No.2	2.5 ng/mL	300 µL No.1 + 300 µL Diluent
No.3	1.25 ng/mL	300 µL No.2 + 300 µL Diluent
No.4	0.625 ng/mL	300 µL No.3 + 300 µL Diluent
No.5	0.312 ng/mL	300 µL No.4 + 300 µL Diluent
No.6	0.156 ng/mL	300 µL No.5 + 300 µL Diluent
No.7	0 ng/mL	300 µL Diluent only (Blank)

The reconstituted standard is for single use only and should not be stored or reused.

5. Reagent Preparation

Biotinylated Antibody: Dilute with Antibody Diluent (1:100). Prepare 30 minutes before use. Do not reuse.

HRP–Avidin Conjugate: Dilute with HRP–Avidin Diluent (1:100). Prepare 30 minutes before use. Do notreuse.

Color Reagent: Mix 9 parts Reagent A with 1 part Reagent B (9:1). Prepare 30 minutes before use andprotect from light.

Assay Procedure

1. Plate Preparation:

Remove the required number of strips from the plate.

Allow them to reach room temperature.

Return unused strips and desiccant to storage at 2–8°C.

2. Blank Wells:

Designate blank wells (optional if dual-wavelength detection is used).Sample and Standard Loading:

Add 100 µL of each standard or sample to the corresponding wells.

Seal with plate film and incubate for 90 minutes at 37°C.

3. Washing (×2):

Wash each well twice with 350 µL of prepared Wash Buffer.

Remove residual liquid completely.

4. Biotinylated Antibody Incubation:

Add 100 µL of diluted Biotinylated Antibody to each well.

Seal and incubate for 60 minutes at 37°C.

5. Washing (×3):

Wash the plate three times with Wash Buffer.

6. HRP–Avidin Incubation:

Add 100 µL of diluted HRP–Avidin solution to each well (omit blank wells).

Seal and incubate for 30 minutes at 37°C.

7. Washing (×5):

Wash the plate five times to remove unbound enzyme.

8. Color Development:

Add 100 µL of prepared Color Reagent to each well (including the blank).

Incubate at 37°C in the dark until the highest standard develops a strong color gradient (≤30 minutes).Reaction **9. Termination:**

Add 100 µL of Stop Solution to each well to terminate the reaction.

Mix gently to ensure uniform color development.

10. Measurement:

Measure absorbance at 450 nm within 10 minutes of adding the Stop Solution.

Calculation

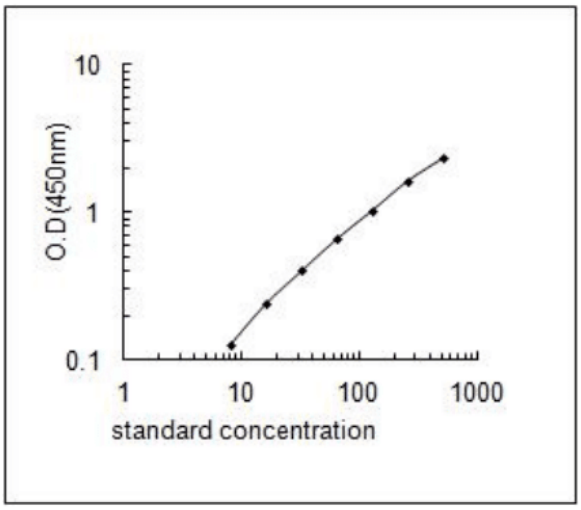
Subtract the average OD value of the blank well from all standard and sample readings.

Generate a standard curve by plotting the concentration of each standard (X-axis) against itscorresponding OD value (Y-axis). Use a smooth curve or regression line to connect the points.

Determine the concentration of each sample by locating its OD value on the standard curve or by usingthe standard curve equation. For best accuracy, it is recommended to use curve-fitting software (e.g.,Curve Expert 1.3) for data analysis.

If the OD value of a sample exceeds the highest point of the standard curve, dilute the sampleappropriately and repeat the assay. Multiply the final calculated concentration by the dilution factor.

Typical Standard Curve



Precision

Intra-Assay Precision (CV): <8%

Inter-Assay Precision (CV): <12%

Detection Range

10 ng/mL – 0.156 ng/mL

Sensitivity

0.05 ng/mL

Recovery

70–110%

Precautions

1. Standard Handling: Reconstituted standards are for single use only and must not be refrozen. Storethe kit at 2–8°C before use.
2. Sample Consolidation: During transport, tubes may need centrifugation to collect material at thebottom. Shake manually or centrifuge at 1000 rpm for 1 minute if necessary.
3. Wash Buffer: Concentrated wash buffer may crystallize slightly. Use a water bath to dissolvecompletely before dilution.
4. Single-Use Standards: Each standard vial is intended for one assay. Always test standards in duplicateor triplicate.
5. Batch Consistency: Do not mix reagents from different kit lots or orders.
6. Reagent Mixing: Ensure reagents are well mixed. For plate reagents, use a micro-oscillator at lowfrequency or manually shake the plate in a circular motion for 1 minute.
7. Chromogen Protection: The color reagent is light-sensitive; avoid direct exposure.
8. Stop Solution Safety: Contains 1M sulfuric acid; handle with care.
9. Sample Addition: Use calibrated pipettes. Add samples quickly (within ~5 minutes). Multi-channelpipettes can help maintain consistent timing.
10. Standard Curve: Prepare a new curve for each assay run. If sample OD exceeds the highest standard,dilute appropriately and adjust calculations according to the dilution factor.
11. Sodium Azide: Samples containing NaN_3 cannot be tested, as it inhibits HRP activity.
12. Plate Washing: For automated washers, inject slightly more than 350 µL per well. Ensure no clogs. Formanual washing, use fresh absorbent material to avoid cross-contamination.
13. OD Reading: Measure absorbance within 10 minutes after adding Stop Solution.
14. Replicates: If wells are duplicated, use the mean value for calculations.
15. Sample Quality: Hemolyzed samples may yield false positives and are not compatible with this kit.
16. Humidity Control: Maintain ~60% humidity during the assay.
17. Temperature Control: Regularly check thermostat calibration to maintain stable 37°C incubation.
18. Sample Dilution: Determine sample concentration based on literature and dilute to fall within the optimal detection range. Document all dilution steps in detail.