STOP SOLN A **Stopping Solution A:**
DSL-10-9780-1 or DSL-10-9780-2

- 1-Plate Kit: One bottle, 15.0 mL
  4-Plate Kit: Two bottles, 27.0 mL
- 0.2 M sulfuric acid.
- Store at 2 to 8°C or room temperature (~25°C) until expiration date.

CONJ DIL **Inhibin A Conjugate Diluent:**
DSL-10-28140-1

- 1-Plate Kit: One bottle, 15.0 mL
  4-Plate Kit: Four bottles, 15.0 mL
- Buffer with BSA, animal serum (goat, mouse), surfactant, and < 1.0% ProClin 300.
- Store at 2 to 8°C until expiration date.

WASHCONC B **Wash Concentrate B:**
DSL-10-9730

- 1-Plate Kit: One bottle, 60.0 mL
  4-Plate Kit: Two bottles, 60.0 mL
- Buffered saline with a nonionic detergent.
- Store at 2 to 8°C or room temperature (~25°C) until expiration date.
- Dilute 25-fold with deionized water prior to use.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Microtitration plate reader capable of absorbance measurement at 450 nm and preferentially capable of dual wavelength correction between 600 and 630 nm
2. Deionized water
3. Precision pipette to deliver 50–100 µL
4. Microtitration plate shaker capable of 500–700 orbital revolutions per minute (rpm)
5. Microtitration plate washer
6. Vortex mixer
7. Absorbent materials for blotting the strips
8. Graph paper for manual data reduction

WARNINGS AND PRECAUTIONS

- **For *in vitro* diagnostic use.**
- Use good laboratory practices.[21]
- Samples and blood-derived products may be routinely processed with minimum risk using the procedure described. However, handle these products as potentially infectious according to universal precautions and good clinical laboratory practices, regardless of their origin, treatment or prior certification.[22] Use an appropriate disinfectant for decontamination. Store and dispose of these materials and their containers in accordance with local regulations and guidelines.

⚠ Caution

- Sodium azide preservative may form explosive compounds in metal drain lines. See NIOSH Bulletin: Explosive Azide Hazard (8/16/76).
  To avoid the possible build-up of azide compounds, flush waste pipes with water after the disposal of undiluted reagent. Sodium azide disposal must be in accordance with appropriate local regulations.
- Inhibin-A Standard A/ Sample Diluent

WARNING
H317 May cause an allergic skin reaction.
P280 Wear protective gloves, protective clothing and eye/face protection.
P333+P313 If skin irritation or rash occurs: Get medical advice/attention.
P362+P364 Take off contaminated clothing and wash it before use.
reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) < 0.05%

- Inhibin-A Standard B, C, D, E, F, G

WARNING
H317 May cause an allergic skin reaction.
P280 Wear protective gloves, protective clothing and eye/face protection.
P333+P313 If skin irritation or rash occurs: Get medical advice/attention.
P362+P364 Take off contaminated clothing and wash it before use.
reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) < 0.05%

- Inhibin-A Control Level I, II

WARNING
H317 May cause an allergic skin reaction.
P280 Wear protective gloves, protective clothing and eye/face protection.
P333+P313 If skin irritation or rash occurs: Get medical advice/attention.
P362+P364 Take off contaminated clothing and wash it before use.
reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) < 0.05%

- Inhibin-A Antibody Enzyme Conjugate Concentrate

WARNING
H316 Causes mild skin irritation.
H317 May cause an allergic skin reaction.
P280 Wear protective gloves, protective clothing and eye/face protection.
P332+P313 If skin irritation occurs: Get medical advice/attention.
P333+P313 If skin irritation or rash occurs: Get medical advice/attention.
P362+P364 Take off contaminated clothing and wash it before use.
3-(N-Morpholino)-2-hydroxy Propane Sulfonic Acid, Sodium Salt 1 - 5%
reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) < 0.05%

- Inhibin-A Sample Buffer A

DANGER
H316 Causes mild skin irritation.
H318 Causes serious eye damage.
P280 Wear protective gloves, protective clothing and eye/face protection.
P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310 Immediately call a POISON CENTER or doctor/physician.
P332+P313 If skin irritation occurs: Get medical advice/attention.
Sodium Lauryl Sulfate 1 - 5%
Tris(hydroxymethyl)– aminomethane 1 - 5%
octylphenoxypoly(ethoxyethanol) 3 - 8%

TEST PROCEDURE

Preparation of Reagents

1. ***Wash Solution:*** Dilute 1 part Wash Concentrate B with 24 parts deionized water. The resulting working strength wash solution is stable for one month at room temperature (~25°C) when stored in a tightly sealed bottle.
2. ***Inhibin A Antibody-Enzyme Conjugate Solution:*** The Inhibin A Antibody-Enzyme Conjugate Concentrate should be diluted at a ratio of 1 part into 50 parts of the Inhibin A conjugate diluent, according to the number of wells used. For an entire plate, pipet exactly 220 µL of the Inhibin A antibody-enzyme conjugate concentrate into 11 mL of the conjugate diluent.

   NOTE: The Inhibin A antibody-enzyme conjugate concentrate should be freshly diluted 10–15 minutes prior to use.
3. ***Microtitration Wells:*** Select the number of coated wells required for the assay. The remaining unused wells should be placed in the resealable pouch with a desiccant. The pouch must be resealed to protect from moisture.

Assay Procedure

Allow all samples and reagents to reach room temperature (~25°C). Mix reagents thoroughly by gentle inversion before use. After reconstitution of reagents, mix thoroughly, avoiding foam. Standards, controls and samples should be assayed in duplicate.

1. Mark the microtitration strips to be used.
2. Pipet 50 µL of the standards, controls and samples to the appropriate wells.
3. Add 50 µL of the Inhibin A Sample Buffer A to each well using a precision pipette.
4. Add 50 µL of the Inhibin A Sample Buffer B to each well using a precision pipette.
5. Incubate the wells, shaking at 500–700 rpm on an orbital microplate shaker, for three hours at room temperature (~25°C).
6. Prepare the antibody-enzyme conjugate solution by diluting the antibody-enzyme conjugate concentrate in the Inhibin A conjugate diluent as described under the "Preparation of Reagents" section of this package insert.
7. Aspirate and wash each well six times with the wash solution using an automatic microplate washer or manually using a precision pipette. Blot and dry by inverting plate on absorbent material.

   NOTE: Use of an automatic microplate washer is strongly recommended. Incomplete washing will adversely affect assay precision. If a microplate washer is not available, follow these steps to wash the plate manually:

   (a) Completely aspirate the liquid from each well

   (b) Dispense 350 µL of the wash solution into each well using a precision pipette

   (c) Aspirate the liquid again

   (d) Repeat steps (b) and (c) five times
8. Add 100 µL of the antibody-enzyme conjugate solution to each well using a precision pipette.
9. Incubate the wells, shaking at 500–700 rpm on an orbital microplate shaker, for one hour at room temperature (~25°C).
10. Aspirate and wash each well six times with the wash solution using an automatic microplate washer. Blot dry by inverting plate on absorbent material.
11. Add 100 µL of the TMB chromogen solution to each well using a precision pipette.

    Avoid exposure to direct sunlight.
12. Incubate the wells, shaking at 500–700 rpm on an orbital microplate shaker, for 15 minutes at room temperature (~25°C).

    NOTE: Be aware that the color may develop more quickly or more slowly than the recommended incubation time depending on the localized room temperature. Visually monitor the color development to optimize the incubation time.
13. Add 100 µL of the stopping solution to each well using a precision pipette.
14. Read the absorbance of the solution in the wells within 30 minutes, using a microplate reader set to 450 nm.

    NOTE: 1) While reading the absorbance of the microtitration well, it is necessary to program the zero standard as a "Blank".

    2) If wavelength correction is available, set the instrument to dual wavelength measurement at 450 nm with background wavelength correction set between 600 and 630 nm.

RESULTS

1. Calculate the mean absorbance for each standard, control or sample.
2. Plot the log of the mean absorbance readings for each of the standards along the y-axis versus log of the inhibin A concentrations in pg/mL along the x-axis, using a linear curve-fit. Alternatively, the data can be plotted linear vs. linear and a smoothed spline curve-fit can be used.
3. Draw the best fitting curve through the mean of the duplicate points.
4. Determine the Inhibin A concentrations of the controls and samples from the standard curve by matching their mean absorbance readings with the corresponding Inhibin A concentrations.
5. Any sample reading higher than the highest standard should be appropriately diluted using Inhibin A Standard A and reassayed.
6. Any sample reading lower than the analytical sensitivity should be reported as such.
7. Multiply the value by a dilution factor, if required.

   NOTE: If the absorbance readings exceed the limitations of the plate reader, a second reading at 405 nm is needed (reference filter between 600 and 630 nm if available). In this case, proceed to construct a second standard curve as above with the absorbance readings of all standards at 405 nm. The concentration of the off-scale samples at 450 nm is then read from the new standard curve. The readings at 405 nm should not replace the on-scale readings at 450 nm.

LIMITATIONS

- The reagents supplied in this kit are optimized to measure inhibin A levels in serum or plasma.
- For assays employing antibodies, the possibility exists for interference by heterophile antibodies in the sample. Samples from individuals which have been regularly exposed to animals or have received immunotherapy or diagnostic procedures utilizing immunoglobulins or immunoglobulin fragments may produce antibodies, e.g. HAMA, that interfere with immunoassays. Additionally, other heterophile antibodies such as human anti-goat antibodies may be present in samples.[24,25] Such interfering antibodies may cause erroneous results. Carefully evaluate the results of patients suspected of having these antibodies.
- If there is evidence of microbial contamination or excessive turbidity in a reagent, discard the vial.
- The Inhibin A ELISA results should be interpreted in light of the total clinical presentation of the patient, including: symptoms, clinical history, data from additional tests, and other appropriate information.

QUALITY CONTROL

- Inhibin A ELISA controls or other commercial controls should fall within established confidence limits.
- The confidence limits for Inhibin A ELISA controls are printed on the control vial labels.
- A full standard curve, plus low and high level controls, should be included in each assay.
- The TMB chromogen solution should be colorless to very light yellow. Development of a blue color may indicate reagent contamination or instability.
- Quality control materials simulate the characteristics of patient samples and are essential for monitoring the system performance of immunochemical assays. Include QC or other commercially available quality control materials that cover at least two levels of analyte. More frequent use of controls or the use of additional controls is left to the discretion of the user based on good laboratory practices or laboratory accreditation requirements and applicable laws. Follow manufacturer's instructions for reconstitution and storage. Each laboratory should establish mean values and acceptable ranges to assure

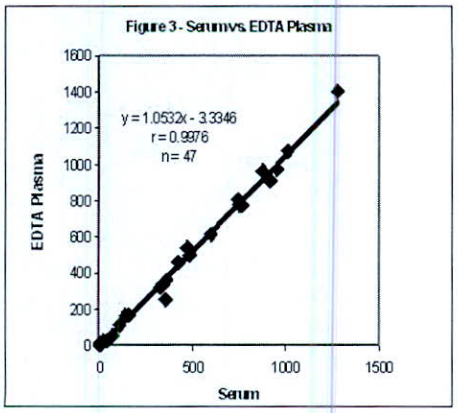
PERFORMANCE CHARACTERISTICS

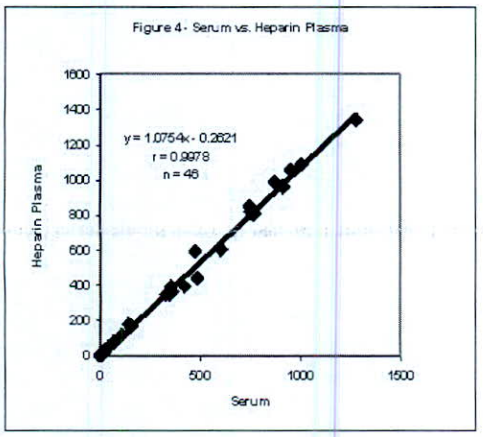
All analytical characteristics are stated in pg/mL. To convert from IU/mL, use the following equation:

1 IU/mL (WHO 91/624) = 26.7 pg/mL

Serum and Plasma Comparisons

Figures 3 and 4 compare inhibin A levels in paired serum vs. EDTA plasma and serum vs. Heparin plasma samples, respectively.





Sensitivity

The theoretical sensitivity, or minimum detection limit, as calculated by interpolation of the mean plus two standard deviations of the 0 pg/mL Inhibin A Standard, is < 5.0 pg/mL.

Precision

The intra-assay precision was determined from the mean of 12 replicates each with five serum samples.

SAMPLE	N	MEAN (pg/mL)	STANDARD DEVIATION (pg/mL)	COEFFICIENT OF VARIATION (%)
1	12	23.2	1.4	6.0
2	12	84.4	3.0	3.6
3	12	211.8	7.6	3.6
4	12	392.1	12.2	3.1
5	12	781.4	23.4	3.0

The inter-assay precision was determined from the mean of two replicates each in two separate runs each day for 20 days with three serum samples.

SAMPLE	N	MEAN (pg/mL)	STANDARD DEVIATION (pg/mL)	COEFFICIENT OF VARIATION (%)
1	40	36.8	2.7	7.3
2	40	197.5	15.4	7.8
3	40	560.1	34.4	6.1

Recovery

Five pairs of serum samples, each containing one low (L) and one high (H) inhibin A sample, were mixed in different proportions as indicated in the table below to determine the percent recovery.

SAMPLE PAIR	LOW/HIGH	EXPECTED (pg/mL)	OBSERVED (pg/mL)	RECOVERY (%)
I	1L / 0H	---	1.4	---
	2L / 1H	235.4	241.0	102
	1L / 1H	352.4	370.9	105
	1L / 2H	469.4	497.6	106
	0L / 1H	---	703.5	---
			MEAN % RECOVERY	105
II	1L / 0H	---	1.1	---
	2L / 1H	272.2	217.0	80
	1L / 1H	407.7	340.4	83
	1L / 2H	543.2	521.9	96
	0L / 1H	---	814.3	---
			MEAN % RECOVERY	86
III	1L / 0H	---	3.5	---
	2L / 1H	312.9	264.3	84
	1L / 1H	467.6	436.1	93
	1L / 2H	622.4	567.5	91
	0L / 1H	---	931.8	---
			MEAN % RECOVERY	90
IV	1L / 0H	---	2.0	---
	2L / 1H	331.1	323.3	98
	1L / 1H	495.6	440.2	89
	1L / 2H	660.1	593.7	90
	0L / 1H	---	989.2	---
			MEAN % RECOVERY	92
V	1L / 0H	---	45.3	---
	2L / 1H	407.7	375.7	92
	1L / 1H	588.9	588.5	100
	1L / 2H	770.1	774.3	101
	0L / 1H	---	1132.5†	---
			MEAN % RECOVERY	98
			OVERALL MEAN % RECOVERY	94

†Inhibin A values above Standard G are extrapolated.

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Printed in U.S.A.
Made in U.S.A.
Revised April 2015

CE 0050

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