

# **DSL Inhibin-A ELSIA Assay Using DSX Microplate Processor**

**Tracking Number CP004**

**Version 1.1**

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## Appendix N

**I. Title**

DSL Inhibin-A ELSIA Assay Using DSX Microplate Processor

**II. Principle**

The Inhibin-A ELSIA assay is an enzymatically amplified two step sandwich immunoassay. Inhibin-A from the sample are incubated in microtiter plate coated an antibody to the  $\beta$  subunit of Inhibin-A. After incubation and a wash step, a second antibody, to the  $\alpha$  subunit of Inhibin-A and labeled with the enzyme horseradish peroxidase (HRP), is added. After a second incubation and wash step, the substrate, tetramethylbenzidine (TMB) is added. An acidic stopping solution is then added and the degree to enzymatic turnover of the substrate is determined by dual wavelength absorbance measurement at 450 and 620 nm. A set of Inhibin-A standards is used to plot a standard curve of absorbance versus Inhibin-A concentration from which the InhibinA concentrations in the unknowns can be calculated.

**III. Specimen Collection and Type**

Serum specimens are collected from pregnant women using the Becton Dickinson SST collection tubes; 4 mL draw in 12 x 75 mm tubes. Collection kits are supplied to the providers by the program.

**IV. Equipment and Supplies**

A. Equipment, supplied by vendor

1. Dynex DSX Microplate Processor
2. Process Controller, Pentium 4, Dell
3. Monitor, Dell, flat panel
4. Printer, Laser Jet 1020, HP
5. Smart-UPS 1500, APC

B. Accessories/Supplies, supplied by vendor

1. Disposable sample tips - 108 tips (12x8)/rack, blue, 4 racks/sleeve
2. Disposable reagent tips - 108 tips/rack, white, 4 racks/sleeve
3. Reagent bottles - 500/box
4. Reagent bottle rack, for reagent preparation
5. Standard/ System Control tubes and caps- 1000/bag
6. Distilled water (or Type 1) reservoir – Position A, 2L
7. Wash Buffer reservoir - 2 on the system, Position B and C, 2L
8. Empty reservoir on system – Position D, not used for Inhibin
9. Liquid Waste bottle
10. Tip waste container
11. Sample caddy – holds 7 sample racks, 2/system
12. Sample racks - 14 positions/rack, 2 sets of 7 racks
13. Reagent/reagent tip rack – 3 rows of 8 positions, 24 total, for reagents, and 2 rows of 41 positions for tips, 2 use to hold the tip disposal chute
14. Tip disposal chute
15. Standard rack – 3 rows of 11 positions

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- C. Supplies Required, but not supplied
1. Disposable pipets - 10, 20 mL
  2. Graduated cylinders - 10, 25 mL

**V. Reagents****A. Reagents, supplied by vendor**

DSL Inhibin-A ELISA reagent kits are stored refrigerated and are used until expiration. The kit contains

1. Microtiter plates – coated with antibody to Inhibin  $\beta$ -subunit, 4 plates
2. Standards - dimeric Inhibin A in fetal bovine serum with a non-mercury preservative, 2 sets, 2 mL for Std A, 1 mL each for C, D, E, F, G (no B), nominal concentrations at 0, 30, 100, 250, 500, and 1000 pg/mL
3. System Controls - dimeric Inhibin A in fetal bovine serum with a non-mercury preservative, 2 sets, 1 mL each, Levels I and II
4. Buffer A – protein based buffer with a non-mercury preservative, 1 bottle, 30 mL,
5. Buffer B – protein based buffer with a non-mercury preservative, 1 bottle, 30 mL
6. Antibody-Enzyme Conjugate Concentrate - anti-inhibin  $\alpha$ -subunit conjugated to horseradise peroxidase with a non-mercury preservative, 4 vials, 600  $\mu$ L
7. TMB Chromogen Solution – tetramethylbenzidine in citrate buffer with hydrogen peroxide, is light sensitive, reacts with water, 1 bottle (dark), 50 mL
8. Stopping Solution – 2 bottles, 27 mL, 0.2 M sulfuric acid
9. Conjugate Diluent – protein based buffer with a non-mercury preservative, 1 bottle, 50 mL
10. Wash Concentrate – buffered saline with a nonionic detergent, 2 bottles, 60 mL

**B. Reagents Required, but not supplied**

1. Distilled water or Type 1 water
2. Ethanol or isopropanol alcohol

**VI. Calibration and Quality Control****A. Calibration**

The calibration is performed using a set of six standards provided with the DSL reagent kit. The standards are run in duplicate on each microtiter plate. Nominal concentrations are 0, 30, 100, 250, 500, and 1000 pg/mL.

**B. System Controls**

A set of two system controls, Levels I and II, is provided in the DSL reagent kit. A single replicate is run following the standards on each microtiter plate.

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## C. Tray Control

Tray control is prepared by and provided by Genetic Disease Laboratory. Tray controls are treated exactly as the maternal serum specimens. A single replicate of the tray control is run as the first sample after System Control II on each microtiter plate. A single replicate of the tray control is run as the last sample of each microtiter plate.

**VII. Procedures**

## A. Accession and Barcoding

1. Refer to the current Accession of Maternal Specimens for Triple Marker Screening Protocol.
2. Refer to the current Automated Dissociated Enhanced Lanthanide Fluoro Immunoassay System for the Determination of hCG, AFP, and uE3 in Maternal Serum Protocol.

## B. Sample Handling

Refer to the current Automated Dissociated Enhanced Lanthanide Fluoro Immunoassay System for the Determination of hCG, AFP, and uE3 in Maternal Serum Protocol.

## C. Warnings and Precautions for Users

1. Some reagents are manufactured from human blood components. The source materials have been tested by approved immunoassays for Hepatitis B surface antigen, anti-Hepatitis C and anti-HIV antibodies and found to be negative. Nevertheless, all blood derivatives, including patient specimens, should be considered potentially infectious and all recommended precautions for the handling of such should be observed. Refer to the U.S. Department of Health and Human Services (Bethesda, Md., USA) publication No. (CDC) 88-8395 on laboratory safety procedures.
2. Avoid contact with reagents containing TMB. TMB is dissolved in a solution that contains tetramethylbenzidine, an irritant to the skin and mucous membranes. TMB is a suspected carcinogen.
3. Avoid contact with reagents containing hydrogen peroxide or sulfuric acid.
4. Wear disposable gloves and safety goggles while handling reagents and maternal serum specimens. Thoroughly wash hands after removing gloves.

## D. Preparation of Reagents

1. Wash Solution
  - a. Pour 60 mL of the wash concentrate into a clean container.
  - b. Dilute with 1500 mL of distilled water.
  - c. Pour diluted wash solution into reservoir in Position B for running Plates 1 and 2.

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- d. Repeat steps a. and b. above and pour into reservoir in Position C for running Plates 3 and 4.
  - e. Keep tightly sealed and use for one week at room temperature.
  2. Diluted Antibody-Enzyme Conjugate Solution
    - a. Add 1 part antibody-enzyme conjugate concentrate to 50 parts of conjugate diluent into a reagent bottle.
    - b. Refer to the Reagent Volumes and Strips Needed by Number of Samples Table for the volume needed for the total number of trays in the run. 1 + 50 is the dilution factor.
    - c. Dilute the concentrate prior to loading into the reagent/reagent tip rack.
    - d. Prepare daily. Once diluted, is stable if 7 hours.
  3. Dispense TQC into plastic tubes.  
Universal TQC for the 4 markers. Refer to AutoDELFI A for the determination of hCG, AFP, and uE3 in Maternal Serum Protocol.
- E. Preparation for Analysis
1. Remove reagents from refrigerator. All reagents must be at room temperature before using. Mix by gentle inversion. For the correct number of plates and strips, refer to the Reagent Volumes Needed by Tray and Strips Table. Use frames saved from used plates for a partial plate.
  2. Turn on power to the DSX instrument. On/Off button is on the right sided of the instrument.
  3. Turn on power to the computer, monitor, and printer.
  4. Load sample tip racks. There are pegs on the surface of the DSX to hold the tip rack and pegs allow the rack be loaded only one way. Use the four positions in the back of the DSX platform. Load a full set of tips in each position. If a tip is missing, the system will try to find the next tip. If system still cannot find a tip, an alarm sounds and an error message appears on the screen and presents the following options:  
  
DSX Pipette Module Error  
Error trying to process sample/standards or controls for immediate transfer  
Failed to detect the fluid surface because the fluid level was not detected  
  
Options  
-Recovery option  
-Manual control  
-Open cover  
-Detail
- Click **Recovery option**. Options are
1. Try to detect the fluid with a new tip

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2. Abort plate test\_1
3. Abort worklist
4. Perform critical abort
5. Load more sample ID “R5001”
6. Manually add this fluid to the target well
7. Abort all remaining wells that require this fluid.

Load a new sample tip rack and select 1. above

5. Load reagent tips into the reagent /reagent tip rack. (Load rack onto DSX when reagent tubes are loaded. See VII.F.) You need 5 tips per tray. If the tip is missing, the system will try again. If system still cannot find a tip, an alarm sounds and an error message appears on the screen and presents the following options:

DXS Pipette Module Error

Error trying to get tip 2 in Reagent Tip Rack 1: Failed to pick up the tip because a tip was not detected

Options

- Recovery option
- Manual control
- Open cover
- Detail

Click **Recovery option**. Options are

1. Try to pick up the tip again
2. Perform critical abort
3. Try another tip
4. Try another tip rack

Fill the reagent tip rack and select 1. above.

6. Fill the reservoir in Position A with distilled water. Refer to the Reagent Volumes Needed by Tray and Strips Table for the volume needed. Remove the electrical connection by pulling at the metal connection and be careful not to get water in the electrical connection. Also be careful not to get water in the black hole on the reservoir. Remove the clear tubing by pressing on the metal tap and pulling the tubing.
7. Fill the reservoir in Position B with wash buffer. Be careful not to pinch the tubing when loading the reservoir. Refer to the Reagent Volumes Needed by Tray and Strips Table for the volume needed. Remove the electrical connection by pulling at the metal connection and be careful not to get water in the electrical connection. Also be careful not to get water in the black hole on the reservoir. Remove the

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tubing by pressing on the metal tap and pulling the tubing.

Reservoir in Position B holds enough only for Plates 1 and 2. Reservoir in Position C is used for Plates 3 and 4. If the run has more than 2 plates, proceed to fill reservoir in Position C.

8. Check all tubings for leaks and creases.

**F. Loading Samples**

1. Load serum specimens and tray controls (TQC) into sample rack from left to right. Dead volume is 350  $\mu$ L. (An adequate specimens is 500  $\mu$ L serum.) Position 1 of the sample rack is the first position on the left and holds 14 specimens.  
**NOTE:** Leading TQC is barcoded T1001 and ending TQC is barcoded T1002 for each tray. The DSX system does not accept duplicate barcodes on the same tray. Print using the Reference icon on the barcode printer. When TQC and MS samples are transferred from the DSX sample racks to the AD sample racks, barcode the TQC tubes using the lot # for TMS TQC.
2. Load serum specimens with the barcode showing in the cut out area of each position. Barcode labels must be at the approximate level of the scanner.
3. Place sample racks onto the sample caddy with position 1 on the left and the barcode in the back.
4. Load no more than 82 specimens (80 maternal serum, 2 TQC) onto the sample racks for one sample rack holder. That is the maximum number of specimens for the first plate (with 12 positions for Stds, 2 for SQC). Only one sample caddy with a maximum of 82 specimens can be loaded onto the DSX at one time.
5. Load the sample caddy onto the DSX. There are pegs on the surface of the DSX to hold the caddy and pegs allow the caddy to be loaded only one way.
6. Proceed to load the second sample caddy with remaining specimens and TQC. DSX provides 2 sample caddies per system with 7 sample racks/caddy.

**G. Loading Reagents**

1. Refer to the Reagent Volumes Needed by Tray and Strips Table for the reagent volume needed.
2. Pour the correct volume of Buffer A into a reagent bottle and place into position 1 the reagent/reagent tip rack. The reagent rack has 24 positions. The first 5 are for Plate 1, the next 5 are for Plate 2, etc. For a 4 plate run, 20 positions are used.
3. Pour the correct volume of Buffer B into a reagent bottle and place into position 2.
4. Place the reagent bottle with the freshly prepared diluted Antibody-Enzyme Conjugate solution into position 3.

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5. Pour the correct volume of TMB Chromogen solution into a reagent bottle and place into position 4.
6. Pour the correct volume of Stopping Solution into a reagent bottle and place into position 5.
7. Proceed to load positions 6 – 10 for the second plate, 11 – 15 for the third plate, and 16 – 20 for the fourth plate.
8. Place the tip disposal chute into the reagent/reagent tip rack. Refer to depiction below.

O (holds the chute)				O (holds the chute)			
Hold reagent tips							
Hold reagent tips							
Buffer A	Buffer B	Conjugate	TMB	Stopping	Buffer A	Buffer B	Conjugate
TMB	Stopping	Buffer A	Buffer B	Conjugate	TMB	Stopping	Buffer A
Buffer B	Conjugate	TMB	Stopping				

(This rack has round holes. This is just to show loading positions.)

9. Load reagent/reagent tip rack onto DSX. Pegs on the surface of the DSX allow the rack to go in only one way.

H. Loading Standards and System Controls

Each 4 plate kit has 2 sets of standards. Each standard can be use for 4 standard curves.

1. Label 8 standard tubes, A, C, D, E, F, G, I and II and the date of expiration.
2. Open Std A and pour the entire content into the vial labeled A (can be used for 4 standard curves).
3. Repeat for Standards C – G and system controls Level I and II.
4. Load the tubes into the standard rack from left to right beginning with the first row.

A	C	D	E	F	G	I	II			

(The standard rack has round holes. This is just to show loading positions.)

5. Repeat with the second set of standards and load into the second row. This is required only if the run has Plate 3 or Plate 3 and Plate 4. The software is set up to sample the standards from the first row for Plates 1 and 2 and from the second row for Plates 3 and 4.

A	C	D	E	F	G	I	II			
A	C	D	E	F	G	I	II			

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4. Load the standard rack onto the DSX. Pegs on the surface of the DSX allow the rack to go in only one way.
5. Use for one week when cap tightly and stored refrigerated.

## I. Analysis

1. Close the cover.
2. Double click **iConsole**.
3. Enter username and password and click **OK**.
4. Click **Create New Assay Run** when the option appears on the screen.
5. View information on the **iConsole Create Assay Run** screen. **Assay Name** defaults to INHID, **Date** defaults to today's date, **Run #** is incremented by the system, **Lot #** defaults to the current lot.  
**NOTE:** The Lot # is entered by the supervisor into the system the day your laboratory begins using the lot. Day to use any Lot # is at the direction of GDL.  
Click **Start**.
6. Read the next screen,  
"Please use DSX Revelation 6.0x to process this run and click view results at the end of the run..."  
**DO NOT** close this window and leave this screen open in the background.
7. Click **Revelation 6.03**.
8. Select the radio button **Connect to DSX (on Port 1)** from the Revelation DSX window and click **Do It**.
9. Enter dynex as the username and password. Click **OK**. The system initializes and brings all components to home position. When completed, the screen shows date, time, serial number of all modules. If each component is performing as expected, "ALL TEST PASSED" is shown for each component. Otherwise, a red error message appears at the top of the screen. If a red error message appears, call DSL for service.
10. Click **File/New Worklist**.
11. Select radio button **Add assays using a new batch of samples**, check **Scan barcodes on new sample batch** and click **OK**.  
**NOTE:** If **Scan barcodes on new sample batch** is not checked, each maternal serum specimen will require you to manually enter the barcode. You don't want that.
12. View the Sample ID Barcode Scanning screen. Click on the circle that represents the last sample, include tray controls and maternal serum specimens, and click **Last Sample**.  
**NOTE:** Each circle representing a sample changes to white. However, the contrast is very poor on the screen and you cannot see the white circle from the background. To see the white circles from the background, you need to look at the screen from the side.
13. Click **Load Sample Caddy**. (See E. Sample caddy is already loaded.)

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14. Click on the green check mark, ✓, on the next screen. Click **OK**.
15. Click **Scan Sample IDs** on the next screen. Step away to avoid the laser beam.  
DSX proceeds to scan the barcodes. As each specimen is scanned, the corresponding white circle turns green.

If an error occurs or a barcode cannot be read, the white circle turns red.

The DSX System does not allow duplicate barcodes. If the reader reads the same barcode a second time, the white circle for the second sample turns yellow.

If there are red circles, read the error message.

“Errors were found while checking the scanned and/or entered sample IDs”.

- a. Click **OK**.
- b. Click **Unload Sample Caddy** and click ✓. DSX unlocks the cover.
- c. Use the red circles on the screen to help find the specimens and fix the problems. Visually check the barcode to be sure it is facing the reader. If the specimen whose barcode cannot be read is under the arm and access to tube is poor, click **Add/Adjust Sample Tubes** on the bottom of the screen to move the arm out of the way for access to the specimen. Click ✓ on the Add/Adjust Sample Tubes screen.
- d. Close the cover and click **Load Sample Caddy**. The message on the screen is  
“Please close the cover when finished and click OK to continue.”  
Close the cover and click **OK**.
- e. Click **Scan Sample IDs** from the “Sample ID Barcode Scanning” screen. DSX proceeds to reread each red circle. If a barcode cannot be read, enter the barcode manually. Click on the red color circle and select **Manual Entry**. Enter the barcode, xxx-xx-xxx/P-year-lab, and click **OK**. The red circle turns to green with a grid and is different from a green circle with a scanned barcode.

If there are yellow circles, read the error message.

“Errors were found while checking the scanned and/or entered sample IDs.

Duplicate sample IDs were found. Replace duplicate labeled sample tubes with unique sample IDs and scan again or manually enter sample IDs. Please fix the indicated error to continue”.

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- f. Click **OK**.
- g. Remove the two samples with the same barcode and replace both positions with a tube filled with tap water barcoded with a reference ID.
- h. Click **Scan Sample IDs**.
- i. Refer to the AutoDELFI A System for the determination of hCG, AFP, and uE3 in Maternal Serum Protocol on how to handle two specimens with the same barcode.

16. View the next screen, “Edit Worklist-Sample Batch”. The screen shows sample rack position number, Sample ID, Test, and Dil (Dil is not used for Inhibin).

- a. Click **New Plate**.
- b. Select **1<sup>st</sup> Inhibin-A Plate\_GDL.asy** and click **Open**. This is the first plate of the Inhibin run.

There is a software glitch and the following screen appears.

Conflicts have occurred ... plates database.

Database version	Imported version
Wash dispense height ...	Wash dispense height ...
.	.
.	.
.	.
Wash sweep ...	Wash sweep ...
Use these settings	Use these setting

Click **Use these setting** for Database version and not Imported version.

The operator can load only one plate at a time. The 2<sup>nd</sup> plate is loaded when the 1<sup>st</sup> plate is in the incubator after addition of Buffer A and Buffer B. The procedure use to load Plates 2, 3, and 4 is identical to the procedure for Plate 1.

The “Edit Worklist” screen now shows <New Plate 1> for Plates and Inhibin A Plate for Assays.

- c. Right click on the **first** cell (open space) below the Test column assay by the first barcode.

Pos	Sample ID	Test

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A1     xxx-xx-xxx | [Click here](#) |

Click **Select # of Samples From Here**. Since the barcodes were scanned, number of specimens is automatically entered on the screen in the field **Please enter the number of samples to be selected**. For a full plate, the number entered is 82, 80 maternal serum and 2 TQC. Click **OK** and the software takes you back to the “Edit Worklist” screen.

17. Click **OK** when the “Edit Worklist” screen appears showing the assay and checked barcodes.
18. Click the down arrow (↓) to view the timeline when completed.
19. Click **Play** (▶) for the system to create the timeline. The screen flashes “Please wait while timeline is being built”.
20. Click **Fast Forward** (▶▶) to start the run. Click the green check mark, √, on the next 2 screens, “Lot Specific Data and Runtime Variable Entry” and “Load Sample Caddy”.  
**NOTE:** This Load Sample Caddy screen is the first time you have access to the red X. The red X is used to cancel the run. It takes you to the beginning of Revelation with iConsole is still open with the same run number.
21. View the “Please load plate” screen with the number of strips needed. Before loading the plate, Enter plate identifier in the top right. Enter mmdyy\_Run #\_Tray #. For example, Tray 2 on Run 3 for test date July 4<sup>th</sup> 2007 has an identifier of 070407\_3\_2. Be sure to include the underscore in the correct place.  
**NOTE:** It is easy to forget this important step. Please do it before loading the plate.
22. Open the cover and load the plate by following the red arrow on the screen to load the plate in the correct position. Click the green check mark, √. Close the cover.  
**NOTE:** If reagents were not loaded for all trays in the run when performing VII.G., do it after clicking √. The drawer goes in and the reagent rack is accessible.  
**NOTE:** On the top of the screen are two icons, one to close the drawer, , and the other to open the drawer, .
23. View the next prompt on the screen and verify that the standard/system control rack and reagent rack are loaded. (See F and G. Racks are already loaded.) Click check mark, √, when completed.  
**NOTE:** When the check mark is clicked, “No” under the “Loaded” column on the screen changes to “Yes” and the next prompt appears at the top of the window screen. As each reagent is highlighted, the volume required for one plate or a partial plate is shown on the screen. **DO NOT USE** the volumes on the screen. Use the Reagent Volumes Needed by Tray and Strips Table.

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24. View the next prompt on the screen and verify that the Wash Buffer and distilled water reservoir are filled. (Reservoir in Position C must be filled if run contains Plates 3 and 4.) Click check mark, ✓, when completed.
25. View the next prompt on the screen and verify that the sample and reagent tips are loaded. Be sure you have full sets of sample tips and each position on the reagent tip rack has a tip. Click check mark, ✓, when completed.
26. Read the next prompt,  
“Ensure tip waste container and washer waste bottle are empty. Please close the cover when finished and click OK to continue.”  
and verify that the tip waste container is empty, waste bottle is empty, and the cover is closed.
27. Click **OK/OK**.
28. Click **Fast Forward** (▶▶|).
29. Observe pipetting of the standards, system controls, and samples. The pipettor has liquid level sensing. If the pipettor cannot find liquid, the system will retry using a new tip. If the system still cannot find liquid, the system stops and requires operator intervention.

An alarm sounds and an error message appears on the screen and presents the following options:

DSX Pipette Module Error  
Error trying to process sample/standards or controls for immediate transfer  
Failed to detect the fluid surface because the fluid level was not detected

Options  
-Recovery option  
-Manual control  
-Open cover  
-Detail

Click **Recovery option**. Options are

1. Try to detect the fluid with a new tip
2. Abort plate mmddy\_run#\_Tray#
3. Abort worklist
4. Perform critical abort
5. Load more sample ID “R5001”
6. Manually add this fluid to the target well
7. Abort all remaining wells that require this fluid.

Find the standard or system control tube that has no liquid. Fill the tube and select 1. Do likewise if it is a reagent that does not have liquid.

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If it is a sample, select 6. above. This event is captured and flagged. Read the message on the screen.

“Please supply the following wells with the fluid specifications described below. When complete close the cover and press OK.”

- a. Use the barcode ID on the screen to find the specimen.
- b. Use the well # with plate identifier on the screen to know which well to add 50 µL of sample.
- c. Open the cover, find the specimen and add to the correct well.
- d. Close the cover and click **OK**.

If insufficient volume for Inhibin, test for TMS if possible and report risk factor using triple markers. Otherwise, call the specimen inadequate.

**NOTE:** The pipettor cannot detect a clot or insufficient volume. Dead volume for this system is 350 µL.

J. Loading 2<sup>nd</sup> Plate

Load the 2<sup>nd</sup> plate when the 1<sup>st</sup> plate goes into the incubator after addition of Buffer A and Buffer B. Follow the same procedure used for loading the 1<sup>st</sup> plate.

**NOTE:** Timing of assay may be adversely effect if Plate 2 is not load immediately after Plate 1 goes into the incubator.

1. Repeat Steps I.9. – I.13. When you click **Load Sample Caddy** in Step I13, the DSX System unlocks the cover.
2. Open the cover and unload the sample caddy for Plate 1 and load the sample caddy for Plate 2. Reagents, standards, system controls, and tips are already loaded with Plate 1. Since this is Plate 2 of the same run, it is not necessary to load a full set of tips. System continues where it left off.  
**NOTE:** Based on the number of samples entered for the run, the system may prompt you to load a full set of samples tips in the first position. Follow the prompt if it is on the screen.
3. Proceed with Step I.14 – I.29. (You do not have to click **Fast Forward**.) For Step 16, select **2nd Inh A Plate-GDL.asy**. For Step 17, use same test date and run number, increment the tray number. For Step 26, you should not have to empty the tip waste container or the waste bottle.

K. Loading Plates 3 or 4

Repeat all the steps in J. Select 3<sup>rd</sup> InhA Plate-GDL.asy or 4<sup>th</sup> InhA Plate-GDL.asy and increment the tray number.

Perform this step immediately after Plate 2 and Plate 3 goes into the incubator.

Maximum number of plates in a run is 4 with 4 incubators. Each time you load a

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sample caddy onto the system, it must contain 82 specimens, a full tray. Only the last plate can be a partial plate. Each sample caddy must have a TQC in the first and last position.

**NOTE:** DSX System allows you to run Trays 1, 2, 3, and/or 4 as a partial tray, but MulticCalc does not. All plates must be a full plate except the last.

**NOTE:** If you plan to reuse the standard tubes, unload as soon as possible, cap tightly, and store in refrigerator.

**L. Abort a Plate or the Run**

If due to a fatal error you wish to abort a tray or the run

1. Click **Pause** and right click anywhere on the timeline.
2. View the screen. Options are
  - Timeline Execution
  - View
  - Settings
  - Manual Control...
  - Open Cover
  - Open Drawer
  - Close Drawer
  - Raise Wash Head
  - Lower Wash Head
  - Abort Plates...
  - Abort All Plates...
3. Click either Abort Plates or Abort all Plates. The Abort Plates screen appears with the plate identifier for each aborted plate.
4. Click Abort.

**M. Shutdown**

1. Click **Stop** (■) when the run is completed. The run is completed when the stop button goes from grey to red and the timeline is no longer on the screen.
2. Open the cover.
3. Remove plates and dump.
4. Click **Tools/DSX manual control/Yes/Drawer/In/Do It/Close** to close the plate drawer.
5. Remove sample racks (if still on the system).
6. Remove reagent bottles and dump the content. Rinse the bottle with distilled water. Allow to dry. Use a bottle for a month and dump.
7. Remove standard/system control tubes (if not already removed). Cap tightly and refrigerate if you plan to use the tube again. Use what is in the tube or dump, do not add or mix standards from a new vial. Two bottles are supplied with 4 plates and each bottle can be used for 4 standard curves.
8. Remove partial sets of sample tips. Collect remaining tips from partial

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sets to make a complete set for use.

9. Close the cover.
10. Perform PWashI to verify that the washer dispenses uniform levels of liquid into each well.
  - a. Click **PwashI**. On the next screen, verify that the radio button for **Add assays using a new batch of samples** is checked and the **Scan barcodes on new sample batch is not selected**.
  - b. Click **OK**.
  - c. Right click in the first cell. Click **Select # of Samples From Here**. There is an automatic entry of 96 for “Please enter the number of samples to be selected”. Click **OK**.  
**NOTE:** <New Plate 1> is entered for Plates and <Daily Morn> is enter for Assay.
  - d. Click **Auto Assign Sample IDs/OK/▶/▶▶** .

If you see an error message on the screen,  
Plate ID Test \_x has already been used.  
Proceed anyway.

Click **Yes**.

On the screen is “Load Sample Caddy 7” screen. Click √. The plate drawer moves to the forward position.

- e. Open the cover and load plate following the red arrow on the “Please load plate for” screen. The plate can be a used Inhibin microtiter plate. Close cover and click √.
  - f. Verify there is sufficient distilled water in the reservoir in Position A when prompt by the “Load with Di H2O” screen. Click √.
  - g. Click **OK** when  
“Ensure tip waste container and washer waste bottle are empty. Please close the cover when finished and click OK to continue” appears on the screen.
  - h. Click **▶▶** . DSX proceeds to dispense liquid into each well.
  - i. Click **Stop (■)**.
  - j. Open cover and remove plate and verify that each well has the same volume of liquid. If not, call DSL for service.
  - k. Return plate to the same position and close the cover.
11. Perform PWashII to verify uniform dryness for each well.
    - a. Click **PWashII**.
    - b. Repeat Steps 10 b. – i. You will see “Load Sample Caddy 8”, you do not need to load plate, and you do not need distilled water. The system proceeds to aspirate each well.
    - c. Open cover and remove plate and verify uniform aspiration and there are no scratches. If not, call DSL for service.
  12. Empty the tip waste container.

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13. Empty the liquid waste container. Remove the electrical connection by pulling at the metal connection. Remove the tubing by pressing on the metal tap and pulling the tubing.
14. Wipe pipette tip with 70% alcohol.
15. Click **Stop** (■) if it is red/x in the upper right corner to close the Revelation Software. Be sure to do this after each run.
16. Turn power off to DSX. Be sure to do this after each run.

## N. Transfer Run to Supervisor's PC

After Revelation is closed, the background iconsole screen is visible. Use this screen to transfer each completed run from the DSX System to the PE Supervisor's PC.

1. Click **Manage Existing Assay Runs** from the iconsole screen.
2. Find the run you want to transfer and highlight the run. Information for each run includes:  
Assay Name, Date, Run #, Created On, Created By, Transferred On, and Transferred By.  
Transferred runs show date of transfer and who transferred the run.
3. Click **View and Transfer**. Use the View drop down menu to view the run. Options to view are: Well IDs, Sample IDs, Results, and Error codes. Results are raw data counts and Error codes are numeric and decoded when transfer to Supervisor's PC.
4. Click **Transfer**. Read the message,  
"Please review all data before transferring the assay run."  
Are you sure you want to transfer this assay run."  
Click **Yes**.  
**NOTE:** If a run was transferred, "Assay run is transferred" appears on the screen.
5. Click **Close/Close**.
6. Exit iConsole.

## O. Review and Release

At the completion of each day's testing, the supervisor at the screening laboratory must review and release the day's data. The DSX data files are put through MultiCalc, the QC software, to calculate results and score controls for determination of run/tray/result status. Software will provide QC plots, trend plots, standard curves, specimen's testing history, and repeat list.

Security is maintained with different access levels to the data base. Staff with Security Level 1 for read only can view the run data using the procedures below but cannot make changes to the data .

1. Refer to TMS Supervisor's Review and Release.

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- a. Enter user name and password. Supervisors performing review and release must logon at Level 2.
  - b. Click **Routine Program/Result Viewer**.
  - c. Select **TMS-Prenatal**.
  - d. Click onto the laboratory icon to begin. Four assays are available for review and release, AFP, hCG, uE3, and Inhibin.
2. Score run/tray according to the following rules:
- a. Score each tray as a separate run and tray since each tray has its own complete standard curve, SQC, and TQC. There is only one rule that applies to the run, assay median. Score the run red if the assay median is outside limits.  
  
If the run has only one partial tray, score the run yellow if the assay median is outside limits.
  - b. Apply Westgard Rules to the 4 control results, 2 SQC and 2 TQC, on each tray.
    - 1) Score the run/tray green to release results if all 4 control results are within 2 SD limits.
    - 2) Score the run/tray green to release results if one of four control results is  $> 2SD$ , either a SQC or a TQC. MutiCalc scores the run/tray yellow as a warning.
    - 3) Score the run/tray red to prevent release of results if two or more control results are  $>2SD$  but within 3 SD, either SQC or TQC.
    - 4) Score the run/tray red to prevent release of results if one or more of the 4 control results are  $>3SD$ .
    - 5) Apply the R4S rule to the pair of TQC results. Score the tray red if the two TQC results exceed 4S.
  - c. Score a run/tray yellow if you want additional review by GDL's QA system.
  - d. Score a run/tray red if you know there was a laboratory error.
3. Score results according to the following rules:
- a. Score an individual Inhibin result red if the result is  $<STD$ . Upon repeat, score the result green and enter fixed comment "confirmed low". If the result is not confirmed, score the result red and repeat again.

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- b. Score an individual Inhibin result red if the result is >STD. Upon repeat, score both the diluted and undiluted results yellow. MultiCalc scores both results red for duplicate barcodes. Enter fixed comment ">STD diluted 1:5" for the diluted result and "confirmed high" for the undiluted result. If results do not confirm, score the results red and repeat again.

**NOTE:** If the concentration is extremely high so that the counts exceed the DSX limits, there is no result for the sample. A concentration is not given by Multicalc and is replaced with "No Value". Multicalc flags the result ">>HH" and scores it red. Repeat as if the result is >STD by testing both diluted and undiluted and scored both results yellow. This happens very infrequently.

- c. Score an individual Inhibin result red if clot was detected for AFP, hCG, or uE3.
- d. Follow the TMS Supervisor's Review and Release Protocol for two specimens with the same barcode due to a barcoding error. The two results with the reference IDs are most likely scored red for <STD. Do not repeat and enter a comment for GDL's QA.

**NOTE:** Liquid handling error messages, e.g., Too little liquid, Liquid surface unstable, etc., are not detected by the DSX Liquid Handling System.

P. Repeat Testing

1. Repeat Inhibin testing for all maternal serum specimens that was prevented from release no later than the next day.
2. Refer to the Automated AutoDELFI A System for the determination of hCG, AFP, and uE3 in Maternal Serum Protocol for the use of the "Repeats Requested Locally" and "Repeats Requested by GDL" lists.
3. Repeat Inhibin testing for a specimen with result >STD. Test diluted and undiluted to confirm result is high.
  - a. Dilute the specimen 1:5. Pipette 100 uL of sample and deliver into a tube. Add 400 µL of Inhibin STD A into the same tube. Mix with the tip by aspirating and dispensing several times.
  - b. Label the tube with the same accession number as the undiluted sample. The accession number must be handwritten.
  - c. Place into sample rack with the diluted sample after the undiluted sample.
  - d. Follow protocol and enter accession number with the "-2" (hyphen 2) extension manually after the lab ID, jjj-cc-sss/P-year-lab-2, when prompted by the DSX system. The "-2" extension is required for the DSX system to flag the result as the diluted result and to prevent having duplicate barcodes on the DSX System. The data

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file in the Supervisor's PC has the correct barcode for the diluted and undiluted result (software has removed the extension), both results flagged as duplicate barcodes, diluted result flagged as the diluted result with an incorrect concentration without application of the dilution factor. The dilution factor of 5 is applied to the result at GDL's QA.

**NOTE:** If you do not wish to enter the entire accession number plus the extension, barcode the diluted tube with the same accession number as the undiluted tube. Since it is a duplicate barcode on the same tray, the circle is yellow for the diluted sample after all barcodes are scanned. Click on the yellow circle and enter the extension -2 after the lab ID since the accession number was already read into the system. To do this the undiluted and diluted tubes must be on the same tray with the diluted after the undiluted sample.

**NOTE:** Both results are scored yellow by your laboratory.

4. Refer to the AutoDELFIA System for the determination of hCG, AFP, and uE3 in Maternal Serum Protocol on how to handle two specimens with the same barcode.

Q. Maintenance

1. Weekly

- a. Wash the 4 reservoirs in Position A, B, C, and D (keep reservoir D clean even if not use) with tap water and rinse with distilled water.
- b. Empty and wash the waste container. Add a small amount of bleach and let it air dry.
- c. Wash the tip waste container.
- d. Run the **Weekend** assay.
  - 1) Clean and rinse wash bottles A, B, C, and D.
  - 2) Fill with distilled water.
  - 3) Click **Weekend** icon.
  - 4) View the Sample Batch Selection screen. Select radio button **Add assays using a new batch of samples** and DO NOT check **Scan barcodes on new sample batch**. Click **OK**.
  - 5) Right click on first cell and click **Select # of Samples From Here** and you see 96 automatically enter. Click **OK**.
  - 6) Click **Auto Assign Sample IDs** and click **OK**.
  - 7) Click play (▶) and fast forward (▶▶|).
  - 8) View the Lot Specific Data and Run time variable Entry screen. Click the green check marks to indicate you are ready. When the prompt ask you to load a plate, you do not have to load a plate, just click **OK**. (A plate is not needed to run this procedure.)

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**NOTE:** The Load Fluid screen shows wash buffer in reservoirs B and C. This is incorrect, reservoirs B and C are filled with distilled water. See P.1.d.2).

- 9) Click stop (■) when completed.
  - e. Turn power off to the PC. Click **Start/Shutdown/OK**.
2. Monthly
    - a. Run Plate Cycle test (18 cycles).
      - 1) Click **Revelation Menu/Tools/DSX Manual Control/Testing/Plate Movement**.
      - 2) Call DSL if the movement of the system is not smooth.
    - b. Run Disk Deragmenter
      - 1) Click **Start/All Programs/Accessories/System Tools/Disk Deragmenter**.
  3. As Needed

Clean the washer head. This is performed when the system fails the PWash I or PWash II test.

    - a. Turn power off to DSX System.
    - b. Remove the big and small tubes from the washer head.
    - c. Lift the washer heard off the arm.
    - d. Use the 2 different size cleaning wires provided and scrape off any crusty deposit from each and every pin of the washer head. The thick wire is for the longer aspirate pins and the thin wire is for the shorter dispense pins.
    - e. Remove the 4 tiny screws, 2 on each side of the washer head, to remove the crust that was scrapped off the pins.
    - f. Hold the washer head vertically under running distilled water or use squeeze bottle filled with distilled water such that the water flows through it.
    - g. Put the 4 small screws back on both sides of the washer head.
    - h. Attach both tubes back to their respective positions on the washer head.
    - i. Place the washer head back on the retaining cradle.
    - j. Run the PWash I and Pwash II test to verify uniform dispensing and aspiration. If it fails again, call DSL.
- R. Inventory

Inhibin kits and supplies have been added to the Perkin Elmer inventory module in Supervisor's PC. Refer to the current AutoDELFIA System for the determination of hCG, AFP, and uE3 in Maternal Serum Protocol.
  - S. Call for Service
    1. Call 1 800 231-7970 to reach DSL's automated answering system.

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2. Press Option 4 for technical support.
3. Press Option 2 for DSX Instrument support.
4. Call the manager of Systems Service & Support, Christopher Santee, on his cell phone if you do not get an answer from a service engineer when you call the 800 number. His cell phone number is given only to the supervisor of each laboratory.

**VIII. Calculations****A. Calibration**

A STD curve is constructed daily for each tray from the duplicate responses for each of the 6 standards. The standard curve is a spline fit line with log concentration on the x-axis and log response on the y-axis.

**B. Patient Results**

At the completion of each run, the file is downloaded to the supervisor's PC where AutoDELFLIA software program automatically converts the instrument readout for each sample to an Inhibin concentration using the full STD curve on each tray.

**IX. Reporting Results**

Results are validated using a quality control program.

**A. Quality Control Program**

The quality control program is used to validate results before reporting. The program is designed to 1) monitor the day to day performances of the DSX and 2) monitor the day to day performance of the assay.

**1. Monitor Performance of the DSX**

- a. Two system controls are provided by DSL to monitor the performance of the DSX. The judgement of whether or not the results for the system controls are acceptable is made using the limits established by Genetic Disease Laboratory in conjunction with the application of the Westgard rules.
- b. The Westgard rules are applied to the two SQC results. By definition, the run is out of control if "3SD 1" (one is outside 3 SD limits), "2SD 2" (two are outside 2 SD), or "R4s" (range of SD exceeds 4 SD). The system issues a warning if "2SD 1" (one is outside 2SD), or if the assay median is outside acceptable limits. These limits and Westgard rules are part of your quality control software.
- c. The analytical run is scored automatically as being in control (green light), out of control (red light), or as a warning (yellow light). Each result which is outside the +/- 2 and 3 SD limits is appropriately flagged.

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- d. System control results can be used to aid in troubleshooting the entire method in the event the results are outside acceptable limits. Specifically, the results of the system controls together with that of tray controls will assist in determining the source of error, such as the degradation of standards and/or reagents, inaccurate dilution of conjugate, instrument malfunction, operator error, or inaccurate sampling.
2. Monitor Performance of the Assay
  - a. A single tray control is provided by the department to monitor the performance of the assay. The tray control is formulated to contain AFP, hCG, uE3, and Inhibin at a clinically appropriate concentration. The judgement of whether or not the results for the tray control are acceptable is made using the limits established by Genetic Disease Laboratory in conjunction with the application of the Westgard rules. The same Westgard rules in use for the SQC are in use for the two TQCs bracketing the patient specimens. These limits and Westgard rules are part of your quality control software.
  - b. The tray is scored automatically as being in control, out of control, or as a warning. Each result is outside the +/- 2 or 3 SD limits is appropriately flagged. If tray control results fall outside the acceptable limits, the supervisor, in consultation with Genetic Disease Laboratory, will determine the source of error, interpret its meaning, and take corrective action.
3. Quality Control Plots

The quality control software will plot results of the system and tray control results showing the mean and acceptable limits. Access the plots using the supervisor's PC.
- B. Repeat Testing

Test must be repeated when results are prevented from release. Refer to the Repeat Testing, Section VII. N.
- C. Completed Worksheet

The completed worksheet is reviewed and released on the supervisor's PC and is automatically transferred overnight, or can be transmitted immediately, to the GDL QA system. Worksheet can be printed with sequence of analysis, well/rack/position number, accession number, counts, concentration, and pertinent flags.

**X. Procedure Notes**

- A. The reagents supplied from DSX are intended for use as an integral unit. Avoid exposure of the reagents to excessive heat or direct sunlight during storage. Do not use kit reagents after the expiration date printed on the kit label.

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- B. The enzyme used as the label is inactivated by oxygen, and is highly sensitive to microbial contamination, sodium azide hypochlorous acid and aromatic chlorohydrocarbons often found in laboratory water supplies.
- C. Frozen patient specimens should be brought to room temperature and mixed by hand. **Mix by gently inverting each sample 3-4 times.** Do not vigorously vortex or mix patient specimens. Foam in the samples may cause sampling errors. Recentrifuge if needed.

**XI. Limitations of Procedure**

A maximum of 320 maternal serum specimens can be tested in one run.

**XII. References**

- A. Inhibin reagent kit, Active Inhibin A ELISA, DSL-10-28100, for in vitro use
- B. DSL a Beckman Coulter Company, Customer Training Guide for DSX Microplate Processor, August 2006

Appendix N

Reagent Volumes Needed by Tray and Strips

# of Plates	1st	2nd	3rd	4th
Assay Buffer A	6 ml	6 ml	6 ml	6 ml
Assay Buffer B	6 ml	6 ml	6 ml	6 ml
Conjugate Concentrate	220 ul	220 ul	220 ul	220 ul
Conjugate Diluent	11 ml	11 ml	11 ml	11 ml
TMB	11 ml	11 ml	11 ml	11 ml
Stop	11 ml	11 ml	11 ml	11 ml


Standards	1 ml	1 ml
Controls	1 ml	1 ml


Positions 1 to 8                      Positions 12 to 19

Di Water (Bottle A)	Filled at the beginning of each day or shift	
Wash Buffer B	1.5 L	1.5 L


Wash Bottle B                      Wash Bottle C

# of Strips	3	4	5	6	7	8	9	10	11	12
Assay Buffer A	2 ml	3 ml	3 ml	3 ml	4 ml	4 ml	5 ml	5 ml	5 ml	6 ml
Assay Buffer B	2 ml	3 ml	3 ml	3 ml	4 ml	4 ml	5 ml	5 ml	5 ml	6 ml
Conjugate Concentrate	60 ul	80 ul	100 ul	120 ul	140 ul	140 ul	160 ul	180 ul	200 ul	220 ul
Conjugate Diluent	3 ml	4 ml	5 ml	6 ml	7 ml	7 ml	8 ml	9 ml	10 ml	11 ml
TMB	3 ml	4 ml	5 ml	6 ml	7 ml	7 ml	8 ml	9 ml	10 ml	11 ml
Stop	3 ml	4 ml	5 ml	6 ml	7 ml	7 ml	8 ml	9 ml	10 ml	11 ml

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**Preventive maintenance Log for DSX Instrument for Inhibin**  
**Month** \_\_\_\_\_ **Year** \_\_\_\_\_

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	
<b>Weekly</b>																																
Wash 4 reservoirs																																
Empty + wash waste container																																
Wash tip waste container																																
Run Weekend																																
Turn off power to PC																																
<b>Monthly</b>																																
Run Plate Cycle Test																																
Run disk defragmentor																																
<b>As Needed</b>																																
Clean washer head																																
<b>Supervisor review</b>																																