

Gel Block Remover	1115
SPIFE Disposable Sample Cups (Deep Well)	3360
SPIFE 20-80 Dispo Cup Tray	3364
SPIFE Disposable Stainless Steel Electrodes	3388

Materials Needed but not Supplied:

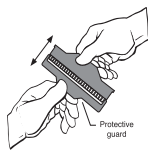
Methanol
Destaining Solution: Mix 75 mL methanol with 25 mL deionized water.

STEP-BY-STEP METHOD

NOTE: If a SPIFE procedure requiring a stain has been run prior to running the Lipoprotein gels, the stainer unit must be cleaned/washed before drying the gel. The new software version 1.20 has an automatic wash cycle prompted by initiation of a test which does not use the stainer unit for staining when the previous test did use the stainer for staining. To avoid delays after staining, this wash cycle should be initiated at least seven (7) minutes prior to the end of staining. To verify the status, press the **TEST SELECT/CONTINUE** button on the stainer until the appropriate test is selected. Place an empty Gel Holder in the stainer unit. If cleaning is required, the "Wash 1" prompt will appear, followed by "Plate out, Holder in" prompts. Press "Continue" to begin the stainer wash. The cleaning process will complete automatically in about 7 minutes. The unit is then ready to dry the gel.

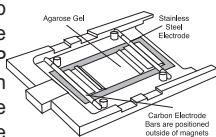
I. Sample Preparation

1. If testing 61-80 samples, remove four disposable Applicator Blade Assemblies from the packaging. If testing fewer samples, remove the appropriate number of Applicator Assemblies from the packaging. Remove the protective guards from the blades by gently bending the protective piece back and forth until it breaks free.
2. Place the four Applicator Blades into the vertical slots in the Applicator Assembly identified as 2, 6, 11 and 15. If using fewer Applicator Blades, place them into any of the four slots noted above. **Please note that the blade assembly will only fit into the slots one way; do not try to force the blade assembly into the slots.**
3. Slide the appropriate number of cup strips into the slots in the Cup Tray.
4. Pipette 75-80 μ L of patient serum or control into each well of the Disposable Cups. If testing less than 61 samples, pipette samples into the row of cups that corresponds with Applicator Blade placement. Cover the tray until ready to use.



II. Gel Preparation

1. Remove the gel from the protective packaging and discard overlay. Using a REP Blotter C, gently blot the entire gel using slight fingertip pressure on the blotter. Remove the blotter.
2. Dispense approximately 2 mL of REP Prep onto the left side of the electrophoresis chamber.
3. Place the left edge of the gel over the REP Prep aligning the round hole on the left pin of the chamber. Gently lay the gel down on the REP Prep, starting from the left side and ending on the right side, fitting the obround hole over the right pin. Use lint-free tissue to wipe around the edges of the plastic gel backing, especially next to electrode posts, to remove excess REP Prep. Make sure no bubbles remain under the gel.
4. Thoroughly wash the electrodes with deionized water before and after each use. Wipe the carbon electrodes with a lint-free tissue. The Disposable Electrode must be patted dry because of the rough surface. Ensure that the endcaps are screwed on tightly. The Disposable Electrode must be replaced after use on 50 gels. Unscrew the endcaps from the old electrode and screw them tightly onto the new electrode.
5. Place a carbon electrode on the outside ledge of the cathode gel block (left side of the gel) outside the magnetic posts.
6. Place a Disposable Stainless Steel Electrode on the outside ledge of the anode gel block (right side of the gel) outside the magnetic posts.
7. Place an Electrode Blotter directly above and below the cathode end of the gel block. Slide the blotters under the ends of the carbon electrode so that they touch the gel block ends. Close the chamber lid.
8. Press the **TEST SELECT/CONTINUE** button located on the Electrophoresis and Stainer sides of the instrument until **LIPO** option appears on the display.



III. Electrophoresis

Using the instructions provided in the Operator's Manual, set up the parameters as follows for the SPIFE 3000:

Electrophoresis Unit

1) No Prompt			
Load Sample 1	00:30	20°C	SPD6

2) No Prompt			
Apply Sample 1	1:00	20°C	SPD6 LOC1
3) No Prompt			
Electrophoresis	20:00	16°C	400 Volt 150mA
4) Remove gel blocks (continue)			
Dry 1	8:00	54°C	
5) No prompt			
END OF TEST			

Stainer Unit

1) No Prompt		
Dry 1	10:00	70°C
2) No Prompt		
END OF TEST		

1. Place the Cup Tray with samples on the SPIFE 3000. Align the holes in the tray with the pins on the instrument.
2. With **LIPO** on the display, press the **START/STOP** button. An option to either begin the test or skip the operation will be presented. Press **START/STOP** to begin. The SPIFE 3000 will apply the samples, electrophorese and beep when completed.
3. Open the chamber lid, remove and dispose of Electrode Blotters. Dispose of blades as biohazardous waste.
4. Using a Gel Block Remover, completely remove the two gel blocks from the gel and discard the gel blocks. Place an electrode on each end of the gel during the drying process to prevent curling. Close the chamber lid.
5. Press the **TEST SELECT/CONTINUE** button to dry the gel.

IV. Visualization of the Lipoprotein Bands

1. Preparation of Staining Solutions

- a. **Stock Stain Solution:** Mix the Lipoprotein Stain in 1 L methanol. Allow to stir overnight and stand for one day. Filter before use. Store at 15 to 30°C.
- b. **Working Stain Solution:** Approximately 5 minutes before use, prepare a working solution of Lipoprotein Stain by adding 5 mL deionized water to 25 mL of stock Lipoprotein stain solution. For best results, add the water to the stain in a dropwise manner while swirling the solution.
- c. **Destaining Solution:** Mix 75 mL methanol with 25 mL deionized water. Mix thoroughly.

2. Recommended Staining Procedure

- a. Place the gel in the REP Gel Staining Dish, agarose side up. Carefully pour 30 mL of the Working Stain Solution directly on the agarose. Wait 4 minutes. Pour off the stain.
- b. Place the gel in Destain Solution for 10 to 20 seconds. Remove excess stain from the sample area using a gloved finger to gently and evenly wipe the gel surface. Pour off the Destain.
- c. Destain the gel again for 10 to 20 seconds. Pour off the Destain Solution.
- d. A brief final water wash may be necessary if trace amounts of background stain remain on the gel. Excessive destaining may cause light or faded bands and/or non-quantitation of control.

3. Drying the Gel

- a. Attach the gel to the SPIFE Gel holder by placing the round hole in the gel mylar over the left pin on the holder and the obround hole over the right pin on the holder.
- b. Place the Gel Holder with the attached gel facing backwards into the stainer chamber.
- c. With **LIPO** on the display, press the **START/STOP** button. An option to either begin the test or skip the operation will be presented. Press **START/STOP** to begin. The instrument will dry the gel.
- d. When the gel has completed the process, the instrument will beep. Remove the Gel Holder from the stainer and you can scan the bands.

V. Evaluation and Quantitation

1. Qualitative evaluation: The SPIFE Lipoprotein Gel may be visually inspected for the presence of bands.
2. Quantitative evaluation: Scan the Lipoprotein Gel in the **QuickScan Touch Plus** using the Fat Red 7B filter and slit 5.

Stability of End Product: Gels to be scanned for the quantitative determination of the bands must be scanned as soon as possible. Gels to be visually inspected for qualitative evaluation only may be kept an indefinite period of time after being processed.

Calibration: A calibration curve is not necessary because relative concentration of the bands is the only parameter determined.

Quality Control:

Lipotrol (Cat. No. 5069) can be used to verify all phases of the procedure and should be used on each gel run. The control should be used as a marker for location of the lipid bands and may also be quantitated to verify the accuracy of quantitations. Refer to the package insert provided with each control for assay values. Additional QC controls may be required for federal, state or local regulations.

proteins). It is accompanied by numerous clinical symptoms and life expectancy is limited. A few cases of familial hypobetalipoproteinemia have been reported. There is some evidence that the mutation is different from that producing abetalipoproteinemia.

LIPOPROTEIN-X

Lipoprotein-X is an abnormal lipoprotein often seen in patients with liver disease. It consists of unesterified (free) cholesterol, phospholipids and protein. It migrates slower than LDL. Because of its particular lipid contents, it stains poorly or not at all with the usual lipid stains and so is not usually detected by standard lipoprotein electrophoretic methods.

PERFORMANCE CHARACTERISTICS

PRECISION

Within Run precision was evaluated by analyzing one sample 80 times on one gel. N = 80

Lipoprotein Fraction	Mean %	SD	CV
Alpha	25.2	0.8	3.1%
Pre-Beta	23.7	0.5	2.1%
Beta	51.1	0.9	1.7%

Between Run: Normal samples were run 80 times each on 5 gels with the following results. N = 400

Lipoprotein Fraction	Mean %	SD	CV
Alpha	24.1	2.1	8.5%
Pre-Beta	22.4	1.6	7.4%
Beta	53.5	3.5	6.5%

CORRELATION

Correlation studies were performed on 64 normal and abnormal specimens. The SPIFE Lipoprotein method was compared to the REP Lipo-30 method resulting in the following linear regression:

N = 64

Y = 0.936X + 2.136

R = 0.957

X = REP Lipo-30

Y = SPIFE Lipoprotein

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