

# SPIFE® Nexus ImmunoFix-9 Procedure in Combination with Template Urine Application

Cat. No. 2409

## INTENDED USE

The SPIFE Nexus ImmunoFix method is a fully automated solution intended for the qualitative identification of monoclonal gammopathies in serum and urine using protein electrophoresis and immunofixation on the SPIFE Nexus system.

For *In Vitro* Diagnostic Use Only.

Rx Only

## SUMMARY

Immunofixation electrophoresis (IFE) has been used for the study of immunoglobulins since 1976.<sup>1,2</sup> It is a two-stage procedure using agarose gel high resolution electrophoresis in the first stage and immunoprecipitation in the second. There are numerous applications for IFE in research, forensic medicine, genetic studies and clinical laboratory procedures. The greatest demand for IFE is in the clinical laboratory where it is primarily used for the detection of monoclonal gammopathies. A monoclonal gammopathy is a primary disease state in which a single clone of plasma cells produces elevated levels of an immunoglobulin of a single class and type. Such immunoglobulins are referred to as monoclonal proteins, M-proteins or paraproteins. Their presence may be of a benign nature or of uncertain significance. In some cases, they are indicative of a malignancy such as multiple myeloma or Waldenstrom's macroglobulinemia. Differentiation must be made between polyclonal and monoclonal gammopathies because polyclonal gammopathies are only a secondary disease state due to clinical disorders such as chronic liver diseases, collagen disorders, rheumatoid arthritis and chronic infections. The SPIFE Nexus IFE method offers many advantages including hands-free operation, ease of interpretation, excellent resolution, reagent conservation and rapid turnaround.

## PRINCIPLE

Proteins are first resolved by electrophoresis. In the second stage, the soluble antigen and antibody are allowed to react. The resultant antigen-antibody complex(es) may become insoluble (as long as the antibody is in slight excess or near equivalency) and precipitate. The precipitation rate depends on the proportions of the reactants, temperature, salt concentration and the pH of the solution. The unreacted proteins are removed by a washing step and the antigen-antibody complex (which might be visible as a white cloudy band in the unstained gel against a dark background) is visualized by staining. The bands in the protein separation are compared with the precipitin bands obtained with immunofixation.

## REAGENTS

### 1. SPIFE IFE-9 Gel

**Ingredients:** Each gel contains agarose in tris-barbital/MOPS buffer with a stabilizer and a preservative.

**WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY. CAUTION: DO NOT INGEST.** The gel contains barbital which, in sufficient quantity, can be toxic.

**Preparation for Use:** The gels are ready for use as packaged.

**Storage and Stability:** The gels should be stored horizontally at room temperature (15 to 30°C) and are stable until the expiration date indicated on the package. The gels must be stored in the protective packaging in which they are shipped. **DO NOT REFRIGERATE OR FREEZE.**

**Signs of Deterioration:** Any of the following conditions may indicate deterioration of the gel: (1) crystalline appearance indicating the agarose has been frozen, (2) cracking and peeling indicating drying of the agarose, (3) bacterial growth indicating contamination, (4) thinning of gel blocks.

### 2. SPIFE Nexus Violet

**Ingredients:** The stain is comprised of 0.2% (w/v) acid violet stain and 10% acetic acid.

**WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY. DO NOT INGEST.**

**Preparation for Use:** The stain is ready for use as packaged.

**Storage and Stability:** The stain solution is stable for one year when stored at 15 to 30°C in a closed container.

**Signs of Deterioration:** The stain should be a homogeneous mixture free of precipitate.

### 3. Citric Acid Destain

**Ingredients:** After dissolution, the destain contains 0.3% (w/v) citric acid.

**WARNING: FOR IN-VITRO DIAGNOSTIC USE. DO NOT INGEST – IRRITANT.**

**Preparation for Use:** Pour 11 L of deionized water into the Destain vat. Add the entire package of destain and mix until completely dissolved.

**Storage and Stability:** Store the destain at 15 to 30°C. It is stable until the expiration date on the package.

**Signs of Deterioration:** Discard if solution becomes cloudy.

### 4. Clear Wash

**Ingredients:** The powder contains anionic and nonionic surfactants, sodium carbonate, enzymes and sodium chloride.

**WARNING: FOR IN-VITRO DIAGNOSTIC USE**

**Preparation for Use:** Dissolve the powder in 8 L of deionized water and mix thoroughly.

**Storage and Stability:** Store the dry powder at 15 to 30°C until the expiration date indicated on the label. The buffer solution should be stored at 15 to 30°C.

**Signs of Deterioration:** The buffer solution should be discarded if it shows signs of bacterial contamination.

### 5. SPIFE Nexus Pipette Wash

**Ingredients:** The buffer solution contains a sodium hydroxide solution.

**WARNING: FOR IN-VITRO DIAGNOSTIC USE. DANGER: CORROSIVE– NEVER PIPETTE BY MOUTH. DO NOT INGEST.**

**Preparation for Use:** The buffer solution is ready for use as packaged.

**Storage and Stability:** The buffer solution should be stored at 15 to 30°C and is stable until the expiration date indicated on the vial.

**Signs of Deterioration:** The buffer solution should be a clear solution.

### 6. SPIFE Nexus IFE Protein Fixative

**Ingredients:** The fixative contains 4.0% sulfosalicylic acid, 6.7% trichloroacetic acid, 0.2% glutaraldehyde and 1.7% guanidine HCl.

**WARNING: FOR IN-VITRO DIAGNOSTIC USE. CORROSIVE – NEVER PIPETTE BY MOUTH. DO NOT INGEST.**

**Preparation for Use:** The fixative is ready for use as packaged.

**Storage and Stability:** The fixative should be stored at 2 to 8°C and is stable until the expiration date indicated on the vial.

**Signs of Deterioration:** The fixative should be a clear solution.

### 7. Antisera to Human IgG, IgA, IgM, Kappa Light Chain and Lambda Light Chain

**Ingredients:** Antisera vials in the kit contain monospecific antisera to human immunoglobulin heavy chains, IgG, IgM, IgA and to human light chains, Kappa and Lambda. The antisera have been prepared in goat. Each vial of antiserum contains a stabilizer and sodium azide as a preservative.

**WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY.** To prevent the formation of toxic vapors, do not mix with acidic solutions. When discarding, always flush sink with copious amounts of water. This will prevent the formation of metallic azides which, when highly concentrated in metal plumbing, are potentially explosive. In addition to purging pipes with water, plumbing should occasionally be decontaminated with 10% NaOH.

**Preparation for Use:** The antisera are ready for use as packaged.

**Storage and Stability:** The antisera should be stored at 2 to 8°C and are stable until the expiration date indicated on the vial.

**Signs of Deterioration:** Extremely cloudy antisera may be indicative of bacterial contamination.

## INSTRUMENT

A SPIFE Nexus analyzer must be used to apply serum samples, electrophorese, apply antisera and fixative, wash, stain, destain, dry and then scan the

gel. The gels may also be scanned on a separate densitometer such as the QuickScan Touch Plus (Cat. No. 1640). Refer to the Operator's Manual for detailed instructions.

## SPECIMEN COLLECTION AND HANDLING

**Specimen:** Fresh serum or urine is the specimen of choice.

**Storage and Stability:** If storage is necessary, samples may be stored covered at 2 to 8°C for up to 72 hours.

### Interfering Factors:

1. Evaporation of uncovered specimens may cause inaccurate results.
2. Use of plasma will cause a fibrinogen band to appear as a distinct narrow band between the beta and gamma fractions. Although fibrinogen does not react with the antisera provided in the kit, fibrinogen may potentially adhere to the gel matrix resulting in nonspecific banding patterns.

## PROCEDURE

**Materials provided:** The following materials needed for the procedure are contained in the SPIFE Nexus IFE-9 Kit. Individual items are not available.

SPIFE IFE-9 Gels (10)	Fixative	1 vial
SPIFE Nexus Violet (1 vial)	IgG	1 vial
Clear Wash (1 pkg)	IgA	1 vial
Citric Acid Destain (1 pkg)	IgM	1 vial
SPIFE Nexus Blotter D (10)	Kappa	1 vial
SPIFE Blotter C (10)	Lambda	1 vial
SPIFE Nexus Pipette Wash (1 vial)		
Serrated Blade Applicator Kit, 18 Sample (30)		

### Materials provided by Helena Laboratories but not contained in the kit:

Item	Cat. No.
SPIFE Nexus Analyzer	1650
QuickScan Touch Plus	1640
SPIFE IFE-9 Dispo Cup Tray	3378
SPIFE IFE-9/15 Dispo Sample Cups	3363
Gel Block Remover	1115
SPIFE Nexus Cassette	2580
SPIFE Nexus Applicator Templates	2570
SPIFE Nexus Applicator Blade Weights	2572
SPIFE Nexus Dispo Stain Cups	2575
Pos ID Barcode Labels for Touch & SPIFE Nexus Systems	1696
REP Prep	3100
SPIFE Nexus Reagent Roller	2583
SPIFE Nexus Ready Run Kit	2582
SPIFE Nexus Antisera Spreader Tips	2574
SPIFE Nexus Carbon Electrode Insert	2576
SPIFE Urine Alignment Guide	3380
SPIFE Urine IFE-9 Accessories	3391
IFE Antisera to IgD	9419
IFE Antisera to IgE	9420
IFE Antisera to Free Kappa	9422
IFE Antisera to Free Lambda	9423

### Materials and Supplies Needed but not Supplied:

0.85% saline

## STEP-BY-STEP METHOD

### I. Sample Preparation

To allow serum and urine samples to be run in combination on the same gel, the SPIFE Nexus analyzer pipettes the diluted serum samples into the sample tray. During serum sample processing, the gel is prepared with urine samples manually applied to the bottom rows of the gel by template application and then placed onboard. The test continues with blade application of the serum samples.

Desired dilutions are operator programmable and may be individually set. Available dilutions are Neat; 1 in 2; 1 in 3; 1 in 4; 1 in 5; 1 in 6; 1 in 7; 1 in 8; 1 in 10; 1 in 12; 1 in 14; 1 in 16; 1 in 18, and 1 in 20 with options ranging from Neat to 1:20. Dilutions are automatically pipetted into the sample cups at a volume of 19 µL per well. See the SPIFE Nexus Operator's Manual for additional instructions. More concentrated samples are more likely to prozone while the more dilute samples may not exhibit desired sensitivity.

### Serum

The SPIFE Nexus automatically samples and dilutes the specimens. By default, the patient serum samples are diluted 1:3 (1 part serum with 2 parts 0.85% saline) for serum protein lanes, and diluted 1:5 (1 part serum with 4 parts 0.85% saline) for immunofix lanes. However, due to desired sensitivity variations, serum samples may also be diluted as follows:

IgG	= 1:5 to 1:10
IgA	= undiluted to 1:5
IgM	= undiluted to 1:5
κ	= undiluted to 1:10
λ	= undiluted to 1:5

The more concentrated samples are more likely to prozone while the more dilute samples may not exhibit desired sensitivity.

### Urine

Due to differences in the running parameters, urine specimens cannot be processed on the SPIFE Nexus in combination with serum samples unless urine specimens are applied using template application. Urine samples should be concentrated if a higher sensitivity is desired. A total protein range of 500-3,000 mg/dL is generally sufficient for optimum sensitivity. The sensitivity is 3 mg/dL for a single band.

Shake samples to homogenize. Centrifuge desired volume at 2000 x g for 5 minutes. Remove supernatant and concentrate to lab specifications per laboratory protocol.

## II. SPIFE Nexus Preparation

- A. Fill the designated bottles with 0.85% saline, deionized water, destain and Clear Wash Solution.
- B. Turn on the SPIFE Nexus. Click on the SPIFE Nexus icon to initialize it.
- C. If this is the first test of the day, prime the instrument according to the instructions in the SPIFE Nexus Operator's Manual.
- D. Serum samples are placed onboard the instrument for sample processing. Load up to 6 uncapped patient serum sample test tubes into a test tube rack. Add one empty and unmarked sample tube into the ninth position of the test tube rack and place rack within the tube transport area. **NOTE: Urine samples are not loaded into the tube transport area. During gel preparation, urine samples will be manually applied to the bottom rows of the gel by template application.**
- E. Open the main door of the instrument and prepare the items onboard the instrument.
  1. Ensure that each of the following items are in their respective onboard storage locations: **Platen Cover** with the Carbon Electrode Insert, **Antisera Spreader Tip**, and **Dryer Cover** with the red sticker toward the back of the instrument.
  2. **Onboard Reagent Chiller**
    - a. Uncap and place the Antisera and Protein Fixative into the labeled onboard Reagent Chiller positions.
    - b. Uncap and place the Saline Diluent into Diluent Well 1, and Pipette Wash into Diluent Well 2 of the Reagent Chiller.
  3. **Sample Cup Tray**
    - a. Prepare the sample cup tray with the appropriate Disposable Sample Cups that correspond to the loaded serum samples. Use only the top row for 3 or fewer serum samples, top and middle rows for up to 6 serum samples. Slide the sample cups into the cup tray.
    - b. Place the cup tray onto the sample tray platform.
  4. **Stain/Reagent Dispenser**
    - a. Fill two Stain Cups each with 400 µL of SPIFE Nexus Violet stain. Place the Stain Cups in the outer two slots of the Stain/Reagent Dispenser. **NOTE: Do not add stain to the middle slot.**
    - b. Place a clean Reagent Roller bar between the hooks on the Stain/Reagent Dispenser.
  5. **Consumables Tray**
    - a. Slide the Consumables Tray forward from its home position.
    - b. Prepare the Applicator Holder
      - (1) Place an IFE-9 Applicator Template on top of the Applicator Holder. Place Applicator Blades in the designated slots corresponding to the sample cups loaded within the sample tray. **NOTE: The Applicator Blades will only fit into the slots in the Applicator Holder one way; do not try to force the Applicator Blades into the slots.**

(2) Place the Applicator Blade Weights on top of the Applicator Blades with the thick side facing the front of the instrument.

c. Prepare the Blotter Holder

(1) Flip the Blotter Holder upside down so the foam surface is upright and place the Blotter Guide around the foam to assist in blotter placement. Locate the double-sided tape on the SPIFE Nexus Blotter D and remove the adhesive backing. Adhere the blotter to the foam surface of the Blotter Holder. Remove the Blotter Guide and place the Blotter Holder back in the designated location within the Consumables Tray with the green dot facing toward the front of the instrument.

d. Slide the Consumables Tray into position in the back of the instrument.

**6. Gel Cassette**

a. Place an empty Gel Cassette on the electrophoresis platen. Make sure that the 2D barcode is located in the upper right corner of the cassette.

F. Close the main door of the instrument.

**III. Sample Loading**

A. Click the Start button on the menu bar. Select the **Serum Immunofixation (IFE) with Urine Template 9 (Acid Violet)** test name from the drop down menu. Ensure the toggles for all Run Processes are set to "Yes" and click the Start Run button. The analyzer will load serum samples.

B. While the SPIFE Nexus automatically loads serum samples, proceed with template application of the urine specimens as follows.

1. Remove the gel from the protective packaging and discard the overlay. Carefully place the gel on the SPIFE Urine Alignment Guide.

2. Using a SPIFE Blotter C, gently blot the entire gel. Discard the blotter.

3. The Urine Templates have been marked with a hole in one corner. Hold the template so that the marked corner is in the lower left position. Align the template pin holes with the pins on the sides of the Alignment Guide and carefully slide the template over the alignment pins until the template contacts the gel surface. Apply slight fingertip pressure across the template, making sure there are no air bubbles between the gel and the template.

4. Pipette 3 µL of each urine sample onto an individual slit of the Urine Template. After pipetting the final sample, use the top half of the clam shell to carefully cover the gel in the Alignment Guide.

5. Allow urine samples to absorb for 5 minutes.

6. After 5 minutes, uncover the gel and gently blot the unabsorbed urine from the Urine Template with a Blotter A-Plus.

7. Carefully remove the blotter and templates and discard as biohazardous waste.

8. Cover the gel until the SPIFE Nexus has completed the serum sample processing.

C. When the SPIFE Nexus has completed serum sample processing, the alarm will sound, the door will unlock, and the following message will be displayed: **Place gel with template-applied urine samples in the gel cassette. Close the door and click 'OK' to continue.**

D. Open the main door of the SPIFE Nexus and place the gel with template-applied urines into the cassette as follows:

1. Remove the top half of the Gel Cassette from the electrophoresis platen.

2. Dispense 2 mL of REP Prep on the platen.

3. Place the left edge of the gel into the bottom of the cassette fitting the round hole over the upper pin and the obround hole over the lower pin. Gently lay the gel down over the REP Prep making sure no bubbles remain under the gel.

4. Place the top half of the Gel Cassette over the gel. Make sure the 2D barcode is located in the upper right corner of the cassette. **Note: Do not use a Positive ID Barcode Label on the gel for this test type.**

E. Close the main door of the instrument.

**IV. Automated Gel Electrophoresis and Scanning**

A. Click the OK button on the onscreen message. The analyzer will apply serum samples, electrophorese, immunofix, wash, stain, destain, dry and

scan the serum samples. For details of Automated Gel Electrophoresis parameters, contact Technical Services.

B. After scanning the serum samples, the Gel Cassette with the finished gel will be located in the scanner port on the front side of the instrument.

1. On the finished gel, record the Gel Identifier number beginning with T000 displayed on the open Worklist. This will serve as the serum scan record locator.

2. If scanning of the urine samples is desired, perform a second scan of the gel as follows:

a. Clean any residual stain from the electrophoresis platen, and ensure that the platen and the back of the finished gel are clean and dry.

b. Lay the cassette containing the finished gel on the platen. Make sure the 2D barcode is located in the upper right corner of the cassette.

c. Close the main door of the instrument.

d. Click the Start button on the menu bar. Select the **Urine Immunofixation (IFE) Scanning for Template Application 9 (Acid Violet)** test name from the drop down menu. Ensure that only the toggle for the Scan process is set to "Yes" and click the Start Run button.

e. The SPIFE Nexus will move the finished gel from the platen to the scanner. After scanning the urine samples, the Gel Cassette with the finished gel will be located in the scanner port on the front side of the instrument.

f. Record on the finished gel the Gel Identifier number beginning with T000 displayed on the open Worklist. This will serve as the urine scan record locator.

C. If gel storage is required, remove and discard the two gel blocks.

D. After every test: discard the used blotters, Applicator Blades, Stain Cups and sample cups as biohazardous waste. Clean any residual stain from the electrophoresis platen, Gel Cassette and the Reagent Roller bar. For daily, weekly, and monthly maintenance reference the SPIFE Nexus Operator's Manual.

**Qualitative Evaluation:** The SPIFE IFE-9 Gel will be automatically scanned. Refer to the QuickScan Touch Plus Operator's Manual for scanning parameters.

**Stability of End Product:** The completed, stained and dried immunofixation gel is stable for an indefinite period of time.

**INTERPRETATION OF RESULTS**

**Normal sample:** No monoclonal present, immunofixation lanes have faint diffused stain or blush of color or variable intensity that reflects the normal distribution of immunoglobins.

**Polyclonal:** No monoclonal present, a diffuse increase in at least one heavy chain and both light chains.

**Monoclonal:** A monoclonal protein is characterized by a well-defined restricted band in a heavy chain lane with a corresponding band in a light chain lane. The monoclonal protein band on the immunofixation pattern will occupy the same migration position and shape as the monoclonal band on the reference protein electrophoresis pattern. The abnormal protein is identified by the corresponding antiserum used. Because of the increased sensitivity of the serum protein procedure. The majority of monoclonal proteins migrate in the cathodic (gamma) region of the protein pattern. However, due to their abnormality, they may migrate anywhere within the protein electrophoresis pattern. When low concentrations of M-protein are present, the immunofixation band may appear on the stained background of the polyclonal immunoglobulin. A stained background may also appear when the M-protein is present along with a large polyclonal increase.

**Other:**

Reaction with light chain antisera only could indicate either a free light chain gammopathy or (rarely) IgD or IgE gammopathy. (See Further Testing)

Reaction with heavy chain antisera may indicate (rarely) heavy chain disease or an atypical light chain.<sup>3</sup>



**Multiple M- Proteins:** On rare occasion biclonal (two M-proteins) or oligoclonal (more than two) patterns may occur.

**Fc fragments** - Proteolysis of the immunoglobulin into Fac and Fc fragments may occur either in vivo or in vitro. The Fc fragments may maintain antigen antibody specificity to a heavy chain only, usually migrate in the alpha-2 area, and appear monoclonal in nature, with no corresponding band in the light chain lane. Fab fragments react with heavy and light chain antisera and are polyclonal in appearance.<sup>4</sup>

**Restricted heterogeneity/Ladder light chains<sup>5</sup>** - A pattern of multiple regularly spaced restricted bands reacting with light chain antisera may be seen in urine with a high concentration of polyclonal light chains.<sup>6</sup>

**Cathodal bands** - An extremely cathodal band present in the SP lane that does not react with antisera is consistent with lysozyme. Elevated lysozyme may be seen in monocytic and monomyelocyte leukemias.<sup>7</sup> For an in-depth discussion of IFE interpretation, call Helena Laboratories toll free and request the free publication "An Immunofixation Tutorial" Book F.

#### Further Testing Required

Specimens containing a band on serum protein electrophoresis suggestive of a monoclonal protein, but which do not react with IgG, IgA or IgM antisera, may require further testing as follows:

1. Serum samples which have a precipitin band with Kappa or Lambda Light Chain Antisera but none corresponding with IgG, IgA or IgM antisera may have a free light chain or they may have an IgD or IgE monoclonal protein. Such sera should be tested with Immunofix IgD and IgE antisera.
2. A CRP band may be detected in patients with acute inflammatory response.<sup>8</sup> CRP appears as a narrow band on the most cathodic end of the high resolution agarose protein electrophoresis pattern. Evaluated Alpha-1 Antitrypsin and haptoglobin (acute phase proteins) are supportive evidence for the presence of a CRP band. Patients with a CRP band will have a positive CRP by latex agglutination or an elevated quantitative CRP.

#### LIMITATIONS

1. Antigen excess will occur if there is not a slight antibody excess or antigen/antibody equivalency at the site of precipitation. Antigen excess in IFE is usually due to a very high level of immunoglobulin in the patient sample. The dissolution of immunoprecipitation is manifested by a loss of protein at the point of highest antigen concentration, resulting in staining at the margins of the band, while leaving the central area with little demonstrable protein stain. In this case, it may be necessary to adjust the protein content of the sample by dilution. Electrophoresing excessive amounts of antigen decreases resolution and requires higher concentrations of antibody. For optimum separation and sufficient intensity for visual detection, care must be taken in adjusting antibody content, sample concentration, time and voltage.
2. Monoclonal proteins may occasionally adhere to the gel matrix, especially cryoglobulins or IgM. These bands will appear in all five antisera reaction areas of the gel. However, where the band reacts with the specific antisera for its heavy chain and light chain, there will be a marked increase in size and staining activity, allowing the band to be identified.
3. An application artifact may appear as a fine clear line (negative space) that may be visible to a faint degree across the entire gel in the beta region. This can on occasion cause the edge of a normal blush to appear slightly blunted.
4. Therapeutic monoclonal antibodies may be used in the treatment of multiple myeloma as well as various other malignancies or medical conditions. If present in sufficient concentration, a humanized therapeutic monoclonal antibody will react with antisera in a manner comparable to a pathologic monoclonal protein.<sup>3,9</sup>
5. Light chains associated with IgA or IgD heavy chains may on rare occasion be difficult to visualize. The structure of some IgA dimers may potentially block the light chain epitopes, decreasing the antisera reaction. This occurs more commonly with lambda than kappa.<sup>5,10</sup>
6. It is possible that not all monoclonals are detected by immunofixation. Not all clinically significant monoclonal gammopathies will display a distinct band detectable by serum protein electrophoresis.<sup>11</sup>

#### PERFORMANCE CHARACTERISTICS

Serum and urine samples containing IgG, IgA, IgM, Kappa light chain and Lambda light chain monoclonal proteins were tested using the SPIFE Touch and SPIFE Nexus instruments. The test results showed complete concordance between instruments.

#### SENSITIVITY

A pathological serum samples with a monoclonal protein at 5.6 g/dL (5600 mg/dL) was serially diluted and the dilutions electrophoresed on the SPIFE IFE-9 gel on the SPIFE Nexus. After visual inspection of the gel, the lowest detectable concentration of a monoclonal protein was between 0.0015 and 0.003 g/L (1.5 and 3.0 mg/dL).

Serial dilutions of a pathological urine sample containing a monoclonal were analyzed on the SPIFE Nexus using template application on the SPIFE IFE-9 gel. The sensitivity was determined to be approximately 0.003 g/L (3 mg/dL) for a single band.

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