TITAN GEL
Immunoelectrophoresis Procedure

Cat. No. 3047

Helena Laboratories

TITAN GEL Immunoelectrophoresis (IEP) is intended for semiquantitative protein determinations by immunoelectrophoresis.

SUMMARY

Immunoelectrophoresis (IEP) combines two techniques, electrophoresis and immunodiffusion. In this two-part procedure, proteins in a serum or urine sample are first separated according to charge by electrophoresis. Then, antisera complimentary to the proteins under study are applied to the plate and allowed to diffuse. When a favorable antigen to antibody ratio exists, a precipitin arc will form on the plate.

IEP is used for the diagnosis and differential diagnosis of monoclonal gammopathies when using serum and urine specimens.[1-6] The method is also used for a number of other purposes, including screening for circulating immune complexes, characterization of cryoglobulinemia and pyroglobulinemia, recognition and characterization of antibody syndromes, and recognition and characterization of the various forms of dysgammaglobulinemias. IEP is a reliable and accurate method for routine protein evaluations, detecting both structural abnormalities and concentration changes.[1-6]

The most common application of IEP is the diagnosis of monoclonal gammopathies. A monoclonal gammopathy is a condition in which a single clone of plasma cells produces elevated levels of a single class and type of immunoglobulin. The elevated immunoglobulin is referred to as a monoclonal protein, M-protein, or paraprotein. Monoclonal gammopathies may indicate a malignancy such as multiple myeloma or macroglobulinemia. The class (heavy chain) and type (light chain) must be established since the patient's prognosis and treatment may differ depending on the immunoglobulin involved. Differentiation must also be made between monoclonal and polyclonal gammopathies.[1-6]

A polyclonal gammopathy is a secondary disease state caused by disorders such as liver disease, collagen disorders, rheumatoid arthritis, and chronic infection. It is characterized by the elevation of two or more (often all) immunoglobulins by several clones of plasma cells. Polyclonal increases are usually twice normal levels.[1-3]

PRINCIPLE

The patient's serum or urine sample and Normal Human Serum Control are electrophoresed on the agarose plate, separating the immunoglobulins according to their electrophoretic mobility. Antisera are then applied to troughs in the plate and allowed to diffuse into the agarose support medium. When a favorable antigen-to-antibody ratio exists, a precipitin arc will form on the plate. Proteins are thus differentiated not only by their electrophoretic mobility, but also by their diffusion coefficient and antibody specificity.

Diffusion is halted by rinsing the plate in 0.85% saline. Unbound protein is washed from the plate by the saline and the antigen/antibody precipitin arcs are stained with a protein sensitive stain. The precipitin arcs formed by the patient sample and the control are compared for a semi-quantitative protein analysis.

REAGENTS

1. **TITAN GEL IEPlate, Cat. No. 3047**
   Ingredients: Plates contain 1% agarose (w/v), in barbital-sodium barbital buffer with 0.1% sodium azide added as a preservative.
   WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY. This product contains sodium azide. Refer to the sodium azide warning.
   Preparation for Use: The plates are ready for use as packaged.
   Storage and Stability: Plates should be stored horizontally at 15 to 30°C and are stable until the expiration date indicated on the label. Store in the protective packaging in which the plates are shipped. **DO NOT FREEZE PLATES OR EXPOSE THEM TO EXCESSIVE HEAT.**
   Signs of Deterioration: The plates should have a smooth, clear agarose surface. Discard the plates if they appear cloudy, show bacterial growth, or if they have been exposed to freezing (a cracked or bubbled surface) or excessive heat (a dried, thin surface).
2. **Antisera for Assay of Immunoglobulins**
   Antiserum to Human IgG, Cat. No. 9232
   Antiserum to Human IgA, Cat. No. 9231
   Antiserum to Human IgM, Cat. No. 9234
   Antiserum to Human IgD, Cat. No. 9249
   Antiserum to Human IgE, Cat. No. 9250
   Antiserum to Human Kappa Light Chain, Cat. No. 9262
   Antiserum to Human Lambda Light Chain, Cat. No. 9257
   Trivalent Antiserum to Human Immunoglobulins (Heavy Chain Specific for IgG, IgA, IgM), Cat. No. 9236
   Antiserum to Human Serum, Cat. No. 9233
   Pentavalent Antiserum (Heavy Chain Specific for IgG, IgA, IgM, IgD, IgE), Cat. No. 9251
   Ingredients: Each vial of antiserum contains the specificity as indicated on the vial. The antisera are prepared in goat. Each vial contains 0.1% sodium azide as a preservative.
   WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY. DO NOT INGEST. The antisera contain sodium azide. Refer to the sodium azide warning.
   Preparation for Use: The antisera are in liquid form and are ready to 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: The antisera should be colorless to light yellow.
3. **IEP Normal Human Serum Control, Cat. No. 9010**
   Ingredients: The control contains pooled normal human serum with 0.1% sodium azide as a preservative.
   WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY. DO NOT INGEST. This product contains sodium azide. Refer to the sodium azide warning. This material has been determined negative for Hepatitis B Antigen (HbsAg) and HIV antibody; however, it should be handled with the same precautions as those observed when handling any human serum.
   Preparation for Use: The control is in liquid form and is ready for use as packaged. Before using, add two drops of Albumin Marker to the control vial.
   Storage and Stability: The control should be stored at 2 to 8°C and is stable until the expiration date indicated on the vial. Stability is not affected by the addition of Albumin Marker.
   Signs of Deterioration: The control should be light yellow and slightly hazy before the addition of marker.
4. **Electra® B1 Buffer, Cat. No. 5016**
   Ingredients: Contains barbital-sodium barbital with sodium azide added as a preservative.
   WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY. DO NOT INGEST. The buffer contains barbital which, in sufficient quantity, can be toxic. Refer to the Sodium azide warning.
   Preparation for Use: Dissolve one package of buffer in one liter of deionized or distilled water. The buffer is ready for use when it is completely dissolved.
   Storage and Stability: The packaged, dry buffer should be stored at room temperature (15 to 30°C) and is stable until the expiration date on the package. Buffer solution is stable for two months at 15 to 30°C.
   Signs of Deterioration: Discard packaged buffer if the material shows signs of dampness or discoloration. Discard buffer solution if it becomes turbid.
5. **Albumin Marker, Cat. No. 9011**
   Ingredients: The Albumin Marker is 0.5% Bromphenol Blue in aqueous solution.
   WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY. DO NOT INGEST.
   Preparation for Use: Add two drops of Albumin Marker to each vial of IEP Normal Human Serum Control. The bromphenol blue binds with the albumin in the control. The tagged albumin allows verification of protein mobility.
   Storage and Stability: The marker should be stored at 15 to 30°C and is stable until the expiration date indicated on the vial.

Signs of Deterioration: Discard if the solution color changes from yellow-brown.

6. **TITAN GEL IEP Stain, Cat. No. 3049**

Ingredients: The stain is Coomassie Brilliant Blue.

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

Preparation for Use: Dissolve the dry stain in 450 mL of reagent alcohol (ethanol). Add 450 mL of deionized or distilled water and 100 mL of glacial acetic acid. Filter before use if necessary. Prepare stain at least one day prior to use in order to obtain good dissolution.

Use 95% ethanol denatured with either methanol or isopropanol. Alcohols denatured with other solvents may cause atypical results.

Storage and Stability: Dry stain should be stored at room temperature (15 to 30°C) and is stable until the expiration date indicated on the package. Stain solution is stable for six (6) months when stored at room temperature (15 to 30°C) in a tightly closed container. The stain can be returned to the bottle and reused.

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

SODIUM AZIDE WARNING

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.

SPECIMEN COLLECTION AND HANDLING

Specimen: Fresh human serum or urine are the specimens of choice. Urine samples should be tested unconcentrated as well as concentrated (10x to 50x) due to the wide range of light chain concentrations.

Patient Preparation: No special patient preparation is necessary.

Interfering Factors:

1. Samples showing evidence of hemolysis should not be used.
2. Inaccurate results may be obtained on specimens left uncovered due to evaporation.
3. Microbial contamination of samples will cause protein denaturation affecting results.
4. Patient age, sex, history and clinical presentation will affect immunoglobulin levels and must be considered.

Storage and Stability: Serum and urine samples should be assayed fresh if possible. Samples may be stored at 2 to 8°C for up to 5 days after collection.

PROCEDURE

Materials Provided: The following materials are provided in the TITAN GEL IEPlate Kit (Cat. No. 3047)

TITAN GEL IEPlate (10)
TITAN GEL Blotter C (1 x 40)
TITAN GEL Blotter E (2 x 40)

Materials Available but Not Provided: The following materials needed for the procedure are available from Helena Laboratories.

Item	Cat. No.
TITAN GEL IEP Stain (1 pkg.)	3049
Electra® B1 Buffer (10 pkg./box)	5016
IEP Normal Human Serum Control (1 x 2.0 mL)	9010
Albumin Marker (1 x 2.0 mL)	9011
Antiserum to Human IgG (1 x 2.0 mL)	9232
Antiserum to Human IgA (1 x 2.0 mL)	9231
Antiserum to Human IgM (1 x 2.0 mL)	9234
Antiserum to Human IgD (1 x 2.0 mL)	9249
Antiserum to Human IgE (1 x 2.0 mL)	9250
Antiserum to Human Kappa Chain (1 x 2.0 mL)	9262
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Trivalent Antiserum to Human Immunoglobulins (IgG, IgA, IgM) (1 x 2.0 mL)	9236
Antiserum to Human Serum (1 x 2.0 mL)	9233
Pentavalent Antiserum (IgG, IgA, IgM, IgD, IgE) (1 x 2.0 mL)	9251
Microdispenser and Tubes (1 to 10 mL)	6210
(1 to 25 mL)	6225
TITAN GEL Chamber	4063
IEP Sponge Wicks, Long (2/pkg.)	9015
Development Weight	5014
Staining Dish	4061

Materials Needed but Not Supplied:

1. Saline Solution (0.85%)
2. Destaining Solution: Mix together 675 mL of 95% ethanol, 675 mL of deionized or distilled water, and 150 mL of glacial acetic acid. Use 95% ethanol denatured with either methanol or isopropanol. Alcohols denatured with other solvents may cause atypical results.
3. Laboratory rotator
4. Power supply capable of providing 100 volts
5. Laboratory drying oven

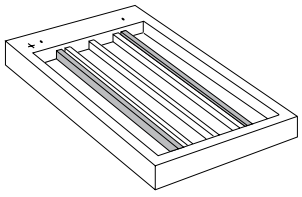
SUMMARY OF CONDITIONS

Plate	TITAN GEL IEPlate
Electra® B_1 Buffer	1 pkg. dissolved in 1.0 L deionized or distilled water
Buffer Volume	100 mL each side
Sample volume	2 µL
Migration distance	35 mm
Electrophoresis time	40 to 50 minutes
Voltage	100 volts
Antisera volume	25 µL
Incubation time	18 to 24 hrs.
Incubation temperature	15 to 30°C
Wash solution	0.85% saline
Six Hour Wash	
Wash time	6 hrs., change every 1 hr.
Drying temperature	60 to 70°C
Drying time	3 to 5 minutes
Quick Wash	
Total wash/press time	35 minutes
Press	4x, 5 minutes each
Wash between presses	3x, 5 minutes each
Drying temperature	60 to 70°C
Drying time	3 to 5 minutes
Stain	TITAN GEL IEP Stain
Staining time	4 minutes
Destaining time	4 to 6 minutes
Drying temperature	60 to 70°C
Drying time	3 to 5 minutes

STEP-BY-STEP METHOD

A. Preparation of TITAN GEL Chamber

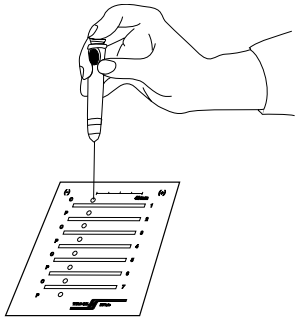
1. Dissolve 1 package Electra® B1 Buffer in one liter deionized or distilled water.
2. Pour 100 mL of buffer into each outer section of the chamber (total of 200 mL of buffer used). The buffer can be reused one time by reversing the polarity of the chamber. The IEPlates must then be placed with the wells on the left side (formerly the anodic side).
3. Place a long IEP Sponge Wick in each buffer-filled compartment. Allow the sponges to become saturated with buffer. Place the sponges against the chamber walls as shown.



4. Cover the chamber until ready to use.

B. Sample Application

1. Add two drops of Albumin Marker to a vial of Normal Human Serum Control.
2. Remove the plate(s) from the protective packaging and save the plastic holder for later use as the incubation chamber.
3. If the wells in the plate contain moisture or the agarose surface appears excessively wet, allow the plate to lay on the counter top for approximately 10 minutes before applying samples. Moisture remaining in the wells can be removed by placing a capillary tube in the well, taking care not to damage the agarose, and allowing the liquid to flow into the tube by capillary action.



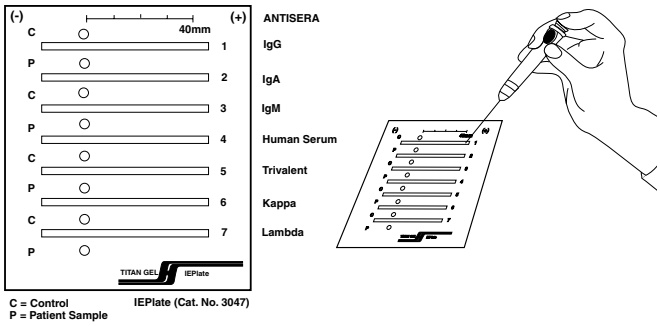
4. Apply 2 µL of the control to wells labeled "C". Apply 2 µL of the patient sample to wells labeled "P". Take care not to damage the wells during sample application.

C. Electrophoresis of the IEPlate(s)

1. Quickly put the plate(s) in the electrophoresis chamber, agarose side down, with the wells toward the cathode (-). Make sure that the agarose is in good contact with the sponge wicks. Two plates may be electrophoresed in one chamber.
2. Put the cover on the electrophoresis chamber and wait 30 to 60 seconds before applying current. This allows the plate(s) to equilibrate with the buffer.
3. Electrophorese the plate(s) at 100 volts for a migration distance of 35 mm. This requires approximately 40 to 50 minutes. Migration distance can be verified by observing the position of the albumin marker.

D. Antisera Application

1. Remove the plate(s) from the chamber and put them on a flat surface, agarose side up.
2. Apply 25 µL of the appropriate antiserum to each trough in the plate. Fill the troughs by placing the tip of a pipette in the end of the trough farthest from the sample well. Holding the pipette in place, slowly depress the plunger and dispense the antiserum into the trough. The antiserum will flow down the trough by capillary action. The troughs easily hold 25 µL of antiserum without overflowing. Severe overfilling may cause antisera to contaminate other troughs, yielding erroneous results.



C = Control
P = Patient Sample
IEPlate (Cat. No. 3047)

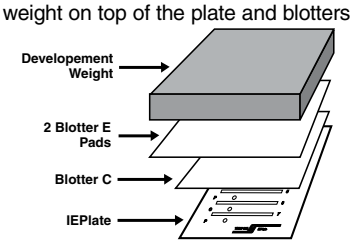
3. Before moving the plates, allow the antisera to absorb for approximately 3 to 5 minutes.
4. Put a moist blotter in the plastic holder(s) saved from Step B.2. Place the plate on the moist blotter and close the lid of the plastic holder(s).
5. Incubate the plate(s) at room temperature (15 to 30°C) for 18 to 24 hours. **The minimum incubation time is 18 hours.** Optimum precipitation will occur between 2 µL of sample and at least 25 µL of antiserum following 18 to 24 hours. If less than 25 µL of antiserum is used, diffuse precipitin arcs will result, interfering with pattern interpretation.

E. Washing and Staining the IEPlates

1. At the end of the incubation period, remove the plate(s) from the plastic holder(s) and put the plate(s) in 0.85% saline. The saline stops the diffusion reaction and washes out unbound protein.
2. Wash and press-dry the plate using the (a) Quick Wash, (b) Six Hour Wash, or the (c) 24 Hour Wash Method.

a. Quick Wash

1. Press the plate dry for 5 minutes as follows: Lay the plate on a flat surface, agarose side up. Place one Blotter C directly on the plate, followed by 2 Blotter E's. Then place a development weight on top of the plate and blotters for 5 minutes.



2. Remove the weight and discard the blotters.
3. Place the plate in a shallow dish containing sufficient 0.85% saline to achieve a level of approximately 1/2 inch. Place the dish on a labortory rotator at slow speed.
4. Wash the plate in 0.85% saline for 5 to 10 minutes.
5. Repeat press and wash steps two more times. After the third wash, press again.

b. Six Hour Wash

1. Place the plate in a shallow dish containing sufficient 0.85% saline to achieve a level of approximately 1/2 inch. Place the dish on a laboratory rotator at slow speed.
2. Wash the plate in 0.85% saline for a minimum of six hours, changing the saline every hour, to remove all unbound protein.
3. Upon completion of washing, press-dry the plate for 5 minutes as follows: Remove the plate from the saline wash and lay it on a flat surface, agarose side up. Place one Blotter C directly on the plate, followed by 2 Blotter E's. Then place a development weight on top of the plate and blotters for five minutes.
4. Remove the weight and discard the blotters.

c. 24 Hour (or overnight) Wash

1. Place the plate in a shallow dish containing sufficient 0.85% saline to achieve a level of approximately 1/2 inch. Place the dish on a laboratory rotator at slow speed and wash overnight.
2. Remove the plate from the saline wash solution and press dry the plate as directed in the other washing methods.

3. Upon completion of the washing/pressing steps, place the plate on a blotter, agarose side up, in a laboratory drying oven at 60 to 70°C for 3 to 5 minutes or until dry. The plate must be completely dry in order to stain and destain properly.
4. Fill a staining dish with prepared TITAN GEL IEP Stain and fill three staining dishes with destaining solution (see Materials Needed but Not Supplied).
5. Place the dried plate(s) in a staining dish for 4 minutes.
6. Remove the plate(s) and place it on a paper towel to drain off surplus stain.
7. Rinse the plate(s) in destain solution to remove excess stain.
8. Repeat Step 7 in the second destaining dish. The background should be clear after this wash; if not, put the plate(s) in the third destaining dish until the background is clear, no more than 2 to 3 minutes. **Do not destain further or the stain in the IgM arc will be lost.** Plates can be restained if necessary.
9. Remove the plate(s) from the destain and put each plate on a blotter agarose side up.
10. Dry the plate(s) in the laboratory drying oven at 60 to 70°C for 3 to 5 minutes or until dry.

Stability of End Product: The stained and dried TITAN GEL IEPlate is stable for an indefinite period of time.

Quality Control: IEP Normal Human Serum Control with Albumin Marker added should be used as a control for each antiserum specificity used.

RESULTS

The formation of a precipitin arc between a well containing test specimen and a trough containing antiserum indicates the presence of the protein specific to the antiserum. The lack of a precipitin arc indicates that a detectable amount of the protein is not present in the test specimen. The size, location, and shape of the precipitin arc, as compared to the control, are indications of the amount of protein in the test sample. IEP is a semiquantitative technique. In general, when protein concentrations are below normal, precipitin arcs are shortened and located farther from the antiserum trough compared to the corresponding arc in the control. When protein concentrations are above normal, precipitin arcs are thicker and located closer to the antiserum trough compared to the control.

Further Testing Required:

A. IgM Typing (IgA)

Interpretation of IgM Light Chain reactions is often difficult due to the umbrella effect of IgG. The following method may be used to depolymerize IgM (19S) into single molecular units (7S), which diffuse through the agarose more rapidly. In rare instances, this may be necessary for IgA typing.

Perform this procedure under a fume hood. The vapor from 2-mercaptoethanol (2-ME) may cause skin irritation. Avoid contact with skin, eyes and clothing.

1. Materials:
   2-mercaptoethanol
   10 µL disposable pipettes
   100 µL disposable pipettes
   Fume hood
2. Make 1:10 dilution, 2-ME to deionized or distilled water.
3. Add 10 µL of diluted 2-ME to 100 µL of patient sample.

4. Assay immediately, running the plate(s) with treated and untreated patient sample in alternate wells.

B. Correction for Antigen Excess

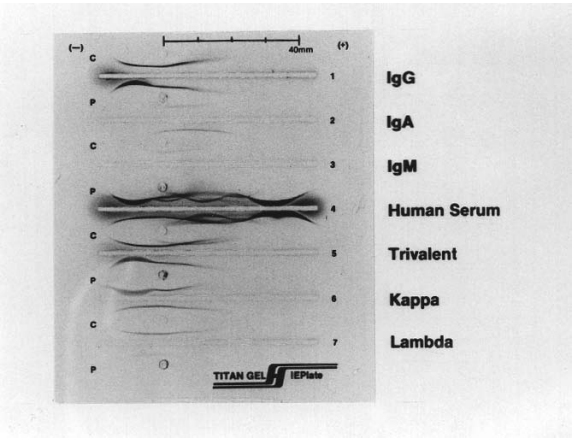
Antigen excess (prozoning) is an incomplete precipitin reaction caused by too high an antigen-to-antibody ratio. Antigen excess should be suspected if a precipitin arc appears to "run" into a trough or if a light chain appears fuzzy when a heavy chain is increased or if an arc appears to be incomplete.

To correct for antigen excess, use one of the following three methods.

1. After incubation but before washing in saline, add an additional 25 µL of antisera to the troughs in question and incubate an additional 18 to 24 hours.
2. Retest the sample using a total of 50 µL of antiserum in the trough(s) in question. First add 25 µL and allow the antiserum to diffuse into the plate, then add the additional 25 µL antiserum.
3. Make a 1:2 dilution of the patient sample with saline and retest.

INTERPRETATION OF RESULTS

A sample immunoglobulin profile is illustrated below. The pattern of precipitin arcs are interpreted comparing the patient sample to the control. In the figure, the patient serum forms a dense, bowed arc against IgG antiserum. There appears to be a diminished IgA level and virtually no IgM in the patient serum when compared to the IgA and IgM in the control. The abnormal IgG band is also visible against both Human Serum and Trivalent antisera. The patient sample reveals a bowed, abnormal kappa arc and a decreased lambda arc. The composite is indicative of an "IgG Monoclonal Gammopathy, Kappa type".



C = Control P = Patient Sample
IgG Monoclonal Gammopathy, Kappa Type

PERFORMANCE CHARACTERISTICS

Serum and urine samples were tested using the TITAN GEL IEPlate system and the TITAN IV IEPlate system to identify abnormals and to classify the M-proteins by class and type. The results from the two systems were identical.

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TITAN GEL Immunoelectrophoresis

TITAN GEL IEPlate Kit **Cat. No. 3047**

TITAN GEL IEPlate (10)
TITAN GEL Blotter C (1 x 40)
TITAN GEL Blotter E (2 x 40)

Other Supplies and Equipment

The following items, needed for the performance of the TITAN GEL IEP Procedure, must be ordered individually.

	Cat. No.
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IEP Normal Human Serum Control (1 x 2.0 mL)	9010
Albumin Marker (1 x 2.0 mL)	9011
Antiserum to Human IgG (1 x 2.0 mL)	9232
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Microdispenser and Tubes (1 to 10 µL)	6210
(1 to 25 µL)	6225
TITAN GEL Chamber	4063
IEP Sponge Wicks, Long (2/pkg.)	9015
Development Weight	5014
Staining Dish	4061

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