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HYDRAGEL 1 IF *Violet Acide / Acid Violet*

Ref. 4301

HYDRAGEL 2 IF *Violet Acide / Acid Violet*

Ref. 4302

HYDRAGEL 4 IF *Violet Acide / Acid Violet*

Ref. 4304

Ref. 4381*

HYDRAGEL 4 IF *Amidoschwarz / Amidoblack*

Ref. 4308

HYDRAGEL 9 IF *Violet Acide / Acid Violet*

Ref. 4309

Ref. 4382**

Masque dynamique / Dynamic mask

IVD

CE

2012/03

INTENDED USE

The HYDRAGEL 1 IF, 2 IF, 4 IF and 9 IF kits are designed for detection of monoclonal proteins in human serum and urine by immunofixation electrophoresis. The kits are used in conjunction with the semi-automated HYDRASYS electrophoresis apparatus. The proteins, separated by electrophoresis on alkaline buffered agarose gels, are incubated with individual antisera that are specific against gamma (Ig G), alpha (Ig A) and mu (Ig M) heavy chains, and kappa (free and bound) and lambda (free and bound) light chains, respectively. After removing the non-reacted proteins, the immunoprecipitates are stained either with acid violet or amidoblack. The electrophoregrams are evaluated visually for the presence of specific reactions with the suspect monoclonal proteins.

Each agarose gel is intended to run :
- one sample in the HYDRAGEL 1 IF kit,
- two samples in the HYDRAGEL 2 IF kit,
- four samples in the HYDRAGEL 4 IF kits,
- nine samples in the HYDRAGEL 9 IF kits.

To accommodate diverse user preferences, the HYDRAGEL 4 IF kits are available either with acid violet or with amidoblack.

For *In Vitro* Diagnostic Use.

NOTE : In this instruction sheet, the name "HYDRASYS" is used for both semi-automated HYDRASYS and HYDRASYS 2 instruments.

PRINCIPLE OF THE TEST

Abnormal bands in serum and urine protein electrophoregrams, primarily those in the beta globulin and gamma globulin zones, are always suspect of being monoclonal proteins (M-proteins, paraproteins, monoclonal immunoglobulins) and therefore, an indication of monoclonal gammopathies. To identify these abnormal bands, the technique of immunofixation is applied.
Immunofixation electrophoresis is a simple technique that allows a protein to be anchored after electrophoresis, *in situ*, by forming an insoluble complex with its antibody. It is easy to perform in four steps and is easy to interpret:

1. Separation of proteins by electrophoresis on agarose gel.
2. Immunofixation (immunoprecipitation) of the electrophoresed proteins - the appropriate electrophoretic migration tracks are overlaid with individual antisera. The antisera diffuse into the gel and precipitate the corresponding antigens when present. The proteins in the reference track are fixed with a fixative.
3. The unprecipitated, soluble proteins are removed from the gel by blotting and washing. Precipitin of the antigen-antibody complex is trapped within the gel matrix.
4. The precipitated proteins are visualized by staining.

To detect and identify the suspected monoclonal component, the sample is simultaneously electrophoresed in six tracks. After the electrophoresis, one track serves as a reference providing a complete electrophoretic pattern of the sample's proteins. The remaining five tracks allow characterization of the monoclonal component from its reaction, or lack of, with antisera against gamma (Ig G), alpha (Ig A) and mu (Ig M) heavy chains, and against free and bound kappa and lambda light chains. The immunofixed bands are then compared with the suspect bands in the reference pattern - the corresponding band should have the same migration position.

REAGENTS AND MATERIALS SUPPLIED IN THE HYDRAGEL 1 IF, HYDRAGEL 2 IF, HYDRAGEL 4 IF AND HYDRAGEL 9 IF KITS

WARNING : See the safety data sheets.

HYDRAGEL 1 IF KIT **HYDRAGEL 2 IF KIT** **HYDRAGEL 4 IF KITS** **HYDRAGEL 9 IF KITS**	**PN 4301** **PN 4302**	**PN 4304** **PN 4309**	**PN 4308**	**PN 4381*** **PN 4382****
Agarose Gels (ready to use)	10 gels	10 gels	10 gels	80 gels
Buffered Strips (ready to use)	10 packs, 2 each	10 packs, 2 each	10 packs, 2 each	80 packs, 2 each
Staining solution diluent (stock solution)			1 vial, 60 mL	
Amidoblack Stain (stock solution)			1 vial, 20 mL	
Acid Violet Stain (stock solution)	1 vial, 75 mL	1 vial, 75 mL		8 vials, 75 mL each
Diluent (ready to use)	1 vial, 3.2 mL	1 vial, 32 mL	1 vial, 32 mL	2 vials, 80 mL each 3 vials, 80 mL each
Fixative Solution (ready to use)				1 vial, 2.9 mL
Mammalian immunoglobulins anti-human gamma heavy chains (ready to use)				1 vial, 2.0 mL
Mammalian immunoglobulins anti-human alpha heavy chains (ready to use)				1 vial, 2.0 mL
Mammalian immunoglobulins anti-human mu heavy chains (ready to use)				1 vial, 2.0 mL
Mammalian immunoglobulins anti-human kappa (free and bound) light chains (ready to use)				1 vial, 2.0 mL
Mammalian immunoglobulins anti-human lambda (free and bound) light chains (ready to use)				1 vial, 2.0 mL
Applicators (ready to use)	1 pack of 10	2 packs of 10 each 3 packs of 10 each	2 packs of 10 each	16 packs of 10 each 24 packs of 10 each
Antisera segments (ready to use)	1 pack of 10	1 pack of 10	1 pack of 10	8 packs of 10 each
Filter Papers - Thin	1 pack of 10	1 pack of 10	1 pack of 10	8 packs of 10 each
Filter Papers - Thick	1 pack of 10	1 pack of 10	1 pack of 10	8 packs of 10 each

(*) HYDRAGEL 4 IF MAXI-KIT *(**) HYDRAGEL 9 IF MAXI-KIT*

NOTE : The fixative solution and the antisera are supplied separately from the kits except for MAXI-KIT (See REAGENTS REQUIRED BUT NOT SUPPLIED).
During transportation, the MAXI-KIT can be kept without refrigeration (15 to 30 °C) for 15 days without any adverse effects on performance.

FOR OPTIMAL RESULTS :
All reagents from the same kit must be always used together and according to the package insert instructions.
PLEASE READ THE PACKAGE INSERT CAREFULLY.

1. AGAROSE GELS

Preparation
Agarose gels are ready to use. Each gel contains : agarose ; buffer solution pH 9.2 ± 0.5 ; additives, nonhazardous at concentrations used, necessary for optimum performance.

Use
Support medium for protein electrophoresis and immunofixation.

Storage, stability and signs of deterioration
Store the gels horizontally in the original protective packaging at room temperature (15 to 30 °C) or refrigerated (2 to 8 °C). They are stable until the expiration date indicated on the kit package and the gel package labels. (The arrow on the front of the kit box must be pointing upwards).
Avoid storage close to a window or to a heat source. Avoid important variation of temperature during storage.
DO NOT FREEZE.
Discard when:
(i) crystals or precipitate form on the gel surface or the gel texture becomes very soft (all these result from freezing the gel),
(ii) bacterial or mold growth is indicated,
(iii) abnormal liquid quantity is present in the gel box (as a result of buffer exudation from the gel due to improper storage conditions).

2. BUFFERED STRIPS

Preparation
Buffered sponge strips are ready to use. Each contains : buffer solution pH 9.1 ± 0.5 ; additives, nonhazardous at concentrations used, necessary for optimum performance.

Use
Buffered strips function as electrophoresis buffer reservoir and ensure contact between the gel and electrodes.

Storage, stability and signs of deterioration
Store the buffered strips horizontally in the original protective packaging at room temperature or refrigerated. (The arrow on the front of the kit box must be pointing upwards).
They are stable until the expiration date indicated on the kit package or buffered strips package label.
DO NOT FREEZE.
Discard buffered strips if the package is opened and the strips dry out.

3. STAINING SOLUTION DILUENT (with PN 4308)

Preparation
The stock staining solution diluent must be used as described in paragraph " AMIDOBLACK STAIN ".
It contains an acidic solution pH ≈ 2.

Use
For the preparation of the amidoblack staining solution.

Storage, stability and signs of deterioration
Store the stock staining solution diluent at room temperature or refrigerated. It is stable until the expiration date indicated on the kit package or staining solution diluent vial labels. DO NOT FREEZE.
Do not add any sodium azide.

4. AMIDOBLACK STAIN (with PN 4308)

Preparation
The amidoblack concentrated stain is a visquous solution which may gelify. The integrity of the stock staining solution is not altered by the increase in viscosity or solidification.
In all cases, to obtain a perfect reconstitution of the stain, we advise you to respect the following procedure:
1. Add 15 mL of stain diluent to the concentrated amidoblack vial.
2. Close carefully the vial.
3. Shake very vigorously the vial during approximately 5 seconds.
4. Pour this solution in the container for staining solution processing.
5. Repeat this step twice, three times if necessary.
6. Pour the remaining diluent in the container and complete the volume to 300 mL with distilled or deionized water.
7. Mix contents of stain cubitainer well for 5 to 10 minutes.

The staining solution is ready to use.

NOTE : An incomplete reconstitution of the stain will lead to an under-evaluation of albumin fraction (low percentage or white hole inside the fraction).

After dilution, the working staining solution contains : acidic solution pH ≈ 2 ; amidoblack ; ethylene-glycol ; additives, nonhazardous at concentrations used, necessary for optimum performance.

Use
For staining gels with electrophoretic protein separations.

IMPORTANT : The staining solution is designed to stain only 10 gels. Change the solution after 10 staining steps.

Storage, stability and signs of deterioration
Store both stock and working staining solutions at room temperature or refrigerated in closed containers to prevent evaporation. Stock staining solution is stable until the expiration date indicated on the kit package or staining vial labels.
Working staining solution is stable for 1 month. Its stability may be extended for 3 months if the working solution is refrigerated. The closed container must be stored refrigerated immediately after each use.
Do not store the working staining solution close to a heat source.

5. ACID VIOLET STAIN (with PN 4301, 4302, 4304, 4309, 4381 and 4382)

Preparation

The vial of the stock acid violet stain to be diluted up to 300 mL with distilled or deionized water.
After dilution, the working stain solution contains : acidic solution pH ≈ 2 ; acid violet ; ethylene-glycol ; additives, nonhazardous at concentrations used, necessary for optimum performance.

Use

For staining gels after protein electrophoresis and immunofixation.

IMPORTANT : The staining solution is designed to stain only 10 gels. Change the solution after 10 staining steps.

Storage, stability and signs of deterioration

Store both stock and working stain solutions at room temperature or refrigerated in closed containers to prevent evaporation. Stock stain solution is stable until the expiration date indicated on the kit package or stain vial labels. Working stain solution is stable for 6 months.

6. DILUENT

Preparation

Diluent is ready to use. It contains : buffer solution pH 7.5 ± 0.5 ; bromophenol blue ; additives, nonhazardous at concentrations used, necessary for optimum performance.

Use

For sample dilution. The bromophenol blue serves as a convenient application and migration marker.

Storage, stability and signs of deterioration

Store the diluent at room temperature or refrigerated. It is stable until the expiration date indicated on the kit package or diluent vial labels.
Diluent must be free of precipitate.

7. ANTISERA AND FIXATIVE PACK (with PN 4381 and 4382)

7.1. ANTISERA

Preparation

Ready to use. All antisera are mammalian, anti-human total immunoglobulins. For easy identification of antisera and as an aid in monitoring their application, the antisera are colored with distinct nonhazardous dyes that match the color of the vial label. When antiserum exhibits a slight turbidity, leave the antiserum vial at room temperature for a minimum of 10 minutes. This should be sufficient to clear the solution ; however, if turbidity remains, this should not affect in any way the immunological reaction. In case of insoluble precipitates, it is recommended to centrifuge antisera for 5 minutes at 3000 rpm.

Use

For immunofixation of the electrophoresed proteins.
Antisera may originate from different animal species. Don't mix two different antisera vials, even with the same specificity, and ALWAYS change the tip of the pipette when changing antiserum vials.

IMPORTANT: In order to avoid any contamination between reagents, be careful to replace the cap on each corresponding vial after each use.

Storage, stability and signs of deterioration

Store the antisera refrigerated (2 to 8 °C). They are stable until the expiration date indicated on the kit package or antisera vial labels.
NOTE: During transportation, the antisera can be kept without refrigeration (15 to 30 °C) for 15 days without any adverse effects on performance.

7.2. FIXATIVE SOLUTION

Preparation

Fixative solution is ready to use. It contains : acidic solution pH ≈ 2 ; additives, nonhazardous at concentrations used, necessary for optimum performance. For an easy identification and as an aid in monitoring its application, the fixative is colored with a nonhazardous dye that matches the color of the vial label.

Use

To fix electrophoretically separated proteins in the reference track (ELP).

IMPORTANT: In order to avoid any contamination between reagents, be careful to replace the cap on each corresponding vial after each use.

Storage, stability and signs of deterioration

Store fixative solution at room temperature or refrigerated. It is stable until the expiration date indicated on the kit package or fixative solution vial labels.
Fixative solution must be free of precipitate.

8. APPLICATORS

Use

Precut, single use applicators for sample application.

Storage

Store the applicators in a dry place at room temperature or refrigerated.

9. ANTISERA SEGMENTS

Use

Single use, colored segments for fixative solution and antisera application onto the gel for immunofixation.

WARNING : Segments with antisera have to be handled with care.

10. FILTER PAPERS - THIN

Use

Single use, thin absorbent paper pads for blotting excessive moisture off the gel surface before sample application.

Storage

Store the thin filter papers in a dry place at room temperature or refrigerated.

11. FILTER PAPERS - THICK

Use

Single use, thick absorbent paper pads for blotting unprecipitated proteins off the gel after immunofixation step.

Storage

Store the thick filter papers in a dry place at room temperature or refrigerated.

REAGENTS REQUIRED BUT NOT SUPPLIED

WARNING : See the safety data sheets.

1. ANTISERA AND FIXATIVE SOLUTION PACK (for 4301, 4302, 4304, 4308 and 4309 kits)

The antisera and fixative solution pack, SEBIA, PN 4315, contains 5 antisera vials and 1 fixative solution vial, 1 mL each. **They are specific for the immunofixation procedure with the dynamic mask.**

1.1. ANTISERA

See previous paragraph 7.1.

1.2. FIXATIVE SOLUTION

See previous paragraph 7.2.

2. DESTAINING SOLUTION

Preparation

Each vial of stock Destaining Solution (SEBIA, PN 4540, 10 vials 100 mL each) to be diluted up to 100 liters with distilled or deionized water. It is convenient to dilute only 5 mL of the stock solution to 5 liters, the volume of the destaining solution container.
After dilution, the working destaining solution contains an acidic solution pH ≈ 2.

Use

For destaining, that is removal of excess and background stain from the gels.
For rinsing of the staining compartment after wash step.
To neutralize the acidity of the destaining solution, pour 15 mL of a 50 % solution of sodium hydroxide into the empty waste container.

Storage, stability and signs of deterioration

Store the stock destaining solution at room temperature or refrigerated. It is stable until the expiration date indicated on the kit package or destaining solution vial labels. Working destaining solution is stable for one week at room temperature in a closed bottle. Do not add any sodium azide.
Discard working destaining solution if it changes its appearance, e.g., becomes cloudy due to microbial contamination.
To prevent microbial proliferation in the diluted destaining solution to be stored more than one week, add 5 µL/dL of ProClin 300.
Working destaining solution added with ProClin is stable in a closed bottle at room temperature or refrigerated until the expiration date indicated on the kit package or destaining solution vial labels.

3. HYDRASYS WASH SOLUTION

Preparation

Each vial of the stock HYDRASYS Wash Solution (SEBIA, PN 4541, 10 vials, 80 mL each) to be diluted up to 5 liters with distilled or deionized water.
After dilution, the working wash solution contains : buffer solution pH 8.7 ± 0.5.

Use

The HYDRASYS wash solution is designed to wash unprecipitated proteins from gels. It also serves for cleaning of the HYDRASYS staining compartment. Use periodically, e.g., if the instrument is used daily, wash the staining compartment weekly.

See the package insert for directions to use.

Storage, stability and signs of deterioration

Store the stock and working wash solutions in closed containers at room temperature or refrigerated. They are stable until the expiration date indicated on the wash solution vial label.
Discard working wash solution if it changes its appearance, e.g., becomes cloudy due to microbial contamination.

4. HYDRAGEL IF SAMPLE DILUENT

The Diluent (SEBIA, PN 4588, 1 vial, 80 mL) is ready to use.
Reagent needed for the automated dilution of samples.
See previous paragraph 6.

5. FLUIDIL

Preparation

Fluidil (SEBIA, PN 4587, 1 vial, 5 mL) is ready to use.

Use

To dilute viscous or turbid samples, e.g., sera containing cryoglobulin or cryogel.

Storage, stability and signs of deterioration

Store at room temperature. It is stable until the expiration date indicated on the Fluidil vial label.
Fluidil must be free of precipitate.

NOTES :

The assays that were performed for the validation of reagents demonstrated that, for the different solutions and using an adapted equipment for the reconstitution volume, a variation of ± 5 % on the final volume has no adverse effect on the analysis.
The distilled or deionized water used to reconstitute solutions, must be free of bacterial proliferation and mold (use a 0.22 µm filter) and have a resistivity higher than 10 Megohms x cm.

EQUIPMENT AND ACCESSORIES REQUIRED BUT NOT SUPPLIED

1. HYDRASYS System SEBIA: HYDRASYS 2 SCAN PN 1200, HYDRASYS 2 PN 1201, HYDRASYS 2 SCAN FOCUSING PN 1202, HYDRASYS 2 FOCUSING PN 1203, HYDRASYS PN 1210 or PN 1211 or HYDRASYS FOCUSING PN 1212.
2. Micropipettor, either manual or automated, such as HYDRAPLUS SEBIA, PN 1216, HYDRAPLUS 2 SEBIA, PN 1217 or ASSIST SEBIA, PN 1218, for an alternative way of loading the sample applicators or antisera segments.
3. Wet Storage Chamber, PN 1270, supplied with HYDRASYS.
4. Container Kit supplied with HYDRASYS.
5. Template guide Bar SEBIA supplied with HYDRASYS.
6. Dynamic mask, SEBIA, PN 1255.
7. Pipettes: 8 µL, 10 µL, 12 µL, 20 µL, 100 µL and 200 µL.

SAMPLES FOR ANALYSIS

Sample collection and storage

Fresh samples are recommended for analysis. Serum and urine must be collected according to established procedures used in clinical laboratory testing. Refrigerate samples (2 to 8 °C) as soon as possible after collection for up to one week. For longer storage periods, keep samples frozen (stable at least one month).
Frozen serum samples with sodium azide, 0.02 g/dL, or frozen urine samples with HEPES 0.1 M (pH 6.75) and/or sodium azide, 0.02 g/dL, are stable for at least 3 months.

IMPORTANT: Avoid boric acid and other acids as preservatives for urine.

Thawed samples may give slight application marks due to protein or lipoprotein denaturation.

Sample preparation

1. *Sera*

   Prepare 1 sample for HYDRAGEL 1 IF, 2 or 4 samples for HYDRAGEL IF 2/4 according to the kit, and 9 samples for HYDRAGEL 9 IF.

   Dilute sera prior to application to prevent prozononing at high level of antigen and mix well.

TRACK	SERUM (µL)	DILUENT (µL)
Ig G Immunofixation track	20	100
ELP reference track and all other immunofixation tracks	30	60

Special cases

- If total immunoglobulin level is > 2 g/dL (hypergammaglobulinemia), it is recommended to use higher dilutions of the samples (except ELP track) to obtain normal immunoglobulins concentration.
- If total immunoglobulin level is < 0.5 g/dL (hypogammaglobulinemia), it is recommended to use lower dilutions of the samples.
- The patient's serum or urine sample may also be tested for Bence Jones proteins ; then, it is recommended to use the "BENCE JONES" migration program.

  The sample should be tested with the IF kit and with anti-kappa free light chains (SEBIA, PN 4370) and anti-lambda free light chains (SEBIA, PN 4371) or with a HYDRAGEL 1, 2 or 4 BENCE JONES kit (SEBIA, PN 4321, 4322 or 4324).

  In this case, dilute the serum in saline or in diluent for immunofixation previously diluted 1/4 (1 part diluent, 3 parts distilled or deionized water) 1/10 for ELP, GAM, K and L tracks (1 part serum, 9 parts diluted diluent or saline) and 1/3 (1 part serum, 2 parts diluted diluent or saline) for Kf and Lf tracks.

  If total immunoglobulin level is < 0.5 g/dL, it is recommended to dilute less the serum sample ; for example, 1/5 for ELP, GAM, K and L tracks, and 1/2 for Kf and Lf tracks.
- After refrigeration or freezing, some sera (particularly those containing cryoglobulin or cryogel) may become viscous or develop turbidity. Such sera might present application problems due to hindered diffusion through the sample applicator teeth. In such case, add 25 µL Fluidil to 75 µL serum and vortex for 15 seconds. Then follow the standard procedure.
- Some monoclonal proteins can polymerize resulting in a "monoclonal fraction" appearing on all immunofixed tracks. In this case (i) prepare 1 % beta-mercaptoethanol (BME, or 2-mercaptoethanol, 2 ME) in Fluidil, (ii) add 25 µL of this reducing solution to 75 µL neat serum, (iii) vortex and wait at least 15 minutes minimum (maximum 30 minutes) and then follow the standard procedure.

2. *Concentrated urines*

   Most urine samples must be concentrated. Apply concentrated urine in all tracks. Concentrate urine (with an appropriate device) to a total protein concentration of ≥ 0.5 g/dL or total Ig of approximately 0.1 g/dL. If the urine protein or Ig concentrations are not known, concentrate 20x - 100x.

   IMPORTANT: Some urines have a high salt content. This can cause a gel deformation during migration and consequently, distortion of the migration profiles. If such a distortion makes interpretation inaccurate, the urine should be dialyzed to remove the salts.
   Diffusion of urine samples into the applicator tips may be hindered when the urine (neat or concentrated) is turbid. It is recommended to remove the particulates by centrifugation (e.g., 10 minutes at 3,000 rpm) or filtration (e.g., 0.45 µm syringe filter).

   Sensitivity in urine testing may be increased by increasing the sample application and antisera incubation time using the "BENCE JONES" migration program. Then, the detection limit corresponds to 1 - 5 mg/dL of monoclonal protein. In this technique, the urines samples may be analyzed unconcentrated or concentrated to improve the sensitivity.

   NOTE: Attempts to increase the test sensitivity by concentrating to a higher degree or indiscriminate concentration of urine samples to a high degree must be considered with caution. The use of overly concentrated urine samples often results in formation of false bands. Such samples may have to be re-run at lower concentrations.

   Special case

   The light chains in urine tend to polymerize and form aggregates. If this compromises the interpretation of the results, the light chain polymers should be de-polymerized: (i) mix 1 volume of BME with 9 volumes of water or saline (e.g., 5 µL BME and 45 µL water or saline), (ii) add 5 µL of the diluted BME to 100 µL urine, mix well, incubate for 10 to 15 minutes and apply without any further sample dilution. Proceed with standard immunofixation or "BENCE JONES" programs.

Samples to avoid

- Avoid plasma samples. Fibrinogen gives a band in the reference track, close to the application point in the ß zone that might be taken for a monoclonal protein.
- Avoid hemolyzed samples.
- Avoid aged, improperly stored urine samples where enzymatic degradation of the proteins might occur.

PROCEDURE

The HYDRASYS system is a semi-automated multi-parameter instrument. The automated steps include processing of HYDRAGEL agarose gels in the following sequence: sample application, electrophoretic migration, incubation with fixative solution and antisera, drying, staining, destaining and final drying. The manual steps include handling samples and gels, application of fixative and antisera and setting up the instrument for operation.
READ CAREFULLY HYDRASYS / HYDRASYS 2 INSTRUCTION MANUAL.

I. MIGRATION SET UP

1. Switch on HYDRASYS instrument.
2. Place one applicator for HYDRAGEL 1 IF or HYDRAGEL IF 2/4 (2 samples), two applicators for HYDRAGEL IF 2/4 (4 samples) or three applicators for HYDRAGEL 9 IF, on a flat surface with the well numbers in the right-side-up position (Fig. 1).
   - Apply 10 µL of properly diluted serum or concentrated urine into the applicator wells as follows. Load each applicator within 2 minutes.

	MIGRATION / IMMUNOFIXATION TRACK					
WELLS No. :	**ELP**	**G**	**A**	**M**	**K**	**L**
HYDRAGEL 1 IF	1	2	3	4	5	6
HYDRAGEL IF 2/4						
SAMPLE N° 1 OR 3	2	3	4	5	6	7
SAMPLE N° 2 OR 4	9	10	11	12	13	14
HYDRAGEL 9 IF						
SAMPLE N° 1, 4 OR 7	1	2	3	4	5	6
SAMPLE N° 2, 5 OR 8	7	8	9	10	11	12
SAMPLE N° 3, 6 OR 9	13	14	15	16	17	18

IMPORTANT: For HYDRAGEL IF 2/4, the wells no. 1, 8 and 15 are not used in this test ; they may be marked with a felt tip pen to avoid filling them with sample by mistake.

- Place each applicator into the wet storage chamber with the teeth up [handle it by the plastic tooth protection frame].
  See wet chamber package insert for further details.
- Let the samples diffuse into the teeth for 5 minutes after the last sample application. For later use (up to 8 hours), keep the entire chamber under refrigeration.

3. Open the lid of the migration module and raise the electrode and applicator carriers.

   WARNING: Never close the lid while the carriers are raised !
4. Select "1 IF SM/DM" migration program for HYDRAGEL 1 IF, "2/4 IF SM/DM" for HYDRAGEL IF 2/4 or "9 IF DM" for HYDRAGEL 9 IF, from the instrument menu (left side of the keyboard).
5. Remove buffered strips from the package ; handle them by the plastic ends. Engage the punched ends of the strip's plastic backing to the pins on the electrode carrier ; the strip's plastic backing must face the carrier (Fig. 2).
6. Unpack the HYDRAGEL plate.
   - Roll quickly and uniformly one thin filter paper onto the gel surface to absorb the excess of liquid. Remove the paper immediately.

     WARNING: Do not leave the filter paper for a too long contact with the gel to avoid its dehydration.
   - Pool 120 µL distilled or deionized water for HYDRAGEL 1 IF or 200 µL for HYDRAGEL IF 2/4 and HYDRAGEL 9 IF, on the lower third of the frame printed on the temperature control plate of the migration module.
   - Place the gel plate (the gel side up) with its edge against the stop at the bottom of the printed frame (Fig. 3).
   - Bend the gel and ease it down onto the water pool. Ensure that no air bubbles are trapped, water is spread underneath the entire gel plate and the gel is lined up with the printed frame (Fig. 3).
7. Lower both carriers down. In this position the buffered strips do not touch the gel. DO NOT FORCE THE CARRIERS ALL THE WAY DOWN.
8. Remove each applicator from the wet chamber. Handle it by the protection frame.
   - Snap off the applicator teeth's protection frame.
   - Place the applicator(s) on the carrier :
   - With HYDRAGEL 1 IF or HYDRAGEL IF 2/4 (2 samples), into position No 6,
   - With HYDRAGEL IF 2/4 (4 samples), into position No 3 and 9,
   - With HYDRAGEL 9 IF, into position No 2, 6 and 10.

   IMPORTANT: The numbers printed on the applicator must face the operator (Fig. 4).

   To improve reproducibility of sample application, always position the applicators on the left side of the carrier.
9. Close the lid of the migration module.
10. Start the procedure immediately by pressing the green arrow "START" key on the left side of the keyboard.

    IMPORTANT: Make sure that the ventilation air inlet on the right side of the instrument is not blocked.

MIGRATION - DESCRIPTION OF THE AUTOMATED STEPS

- The two carriers are lowered so that buffered strips and applicator(s) contact the gel surface.
- Sample applicator carrier rises up.
- Migration is carried out under 10 W constant for HYDRAGEL 1 IF and under 20 W constant for HYDRAGEL IF 2/4, until 42 Vh accumulated (for about 9 minutes) and under 20 W constant until 36 Vh accumulated (for about 7 minutes) for HYDRAGEL 9 IF, at 20 °C controlled by Peltier effect.
- The electrode carrier rises to disconnect the electrodes.
- An audible beep signals that the migration module lid unlocks. The following message is displayed on the screen: "⇩ AS" / "⇩ ANTISERA".

NOTE: The migration module lid remains locked during all migration steps.

II. IMMUNOFIXATION SET UP

The dynamic mask contains a colored reference guide for reagent application, an antisera segment, a segment holder, a dynamic mask guide and a length-reducing device (Fig. 5).
During the migration, assemble the dynamic mask as follows :

1. Place the dynamic mask guide on a flat surface.

   IMPORTANT : For one and two samples analysis, it is necessary to position a length-reducing device on the dynamic mask guide.
2. Set up an antisera segment on the segment holder (Fig. 6) :
   - Tilt the antisera segment at a 45° angle and position it against the plastic springs of the segment holder.
   - Pull apart the two elements and pivot the segment to fix it into the notches of the segment holder.

   WARNING : Be sure the segment is correctly positioned on the holder : the pins at ends of the segment must be blocked into the notches of the holder.

3. Set up the holder with the segment on the dynamic mask guide (Fig. 7). Then, put the colored reference guide for reagent application, corresponding to the assay being run, on the segment holder in front of the segment wells (Fig. 8).
4. Apply reagents as follows:
   - 6 wells antisera segment for HYDRAGEL 1 IF : 8 µL per well,
   - 15 wells antisera segment for HYDRAGEL IF 2/4 : 8 µL per well for 2 samples analysis,
     12 µL per well for 4 samples analysis,
   - 18 wells antisera segment for HYDRAGEL 9 IF : 8 µL per well.

TROUGH	REAGENT	COLOR
ELP	fixative solution	yellow
G	anti-gamma heavy chain antiserum	pink
A	anti-alpha heavy chain antiserum	dark blue
M	anti-mu heavy chain antiserum	yellow green
K	anti-kappa light chain (free & bound) antiserum	light green
L	anti-lambda light chain (free & bound) antiserum	light blue

NOTE: Reagents are colored and the colors are shown on the colored reference guide to facilitate correct antisera pipetting.

IMPORTANT : For HYDRAGEL IF 2/4, do not use wells of antisera segment in position 1, 8 and 15.

- Aspirate reagents avoiding any air bubbles in the pipette tip.
- Apply the reagents (Fig. 9) :
- Hold pipette at an angle and rest its tip lightly at the side of the well.
- Inject the drop of reagent into the well without touching the bottom of the well.

5. Remove the colored reference guide.

III. IMMUNOFIXATION

1. Open the migration module lid.
2. Remove the sample applicator(s) and discard.
3. Raise both carriers, remove the buffered strips by their plastic ends and discard.
   - Remove both carriers.
   - Wipe electrodes with soft wet tissue.
   - Leave the gel in place in the migration module.
4. Set up the dynamic mask for reagent application as follows (Fig. 10):
   - Position the mask guide on the anchoring clip (the guide may stay in the migration module all the time).
   - Hold the dynamic mask by the tab and position it into the guide with the notches aligned with the marks.
   - Lower the dynamic mask onto the plate of HYDRASYS.

   IMPORTANT : Adjust the dynamic mask position for perfect alignment between electrophoretic profiles and wells of the mask.
5. Place the segment holder at the lowest point on the mask guide, facing the operator.
   Hold the segment holder by the handle situated on its right and press on the central pressure point such that the antisera segment contacts the gel. Release the pressure; then, reagents will spread under each track (Fig. 11).
6. Immediately, using the segment holder handle, move the segment slowly but steadily up and down the entire length of the gel to apply the reagents. Application should take approximately 5 seconds (Fig. 12).
   WARNING : During this step, hold the mask only by the segment holder handle. Avoid touching the guide. Don't re-press on the pressure point as this may result in cross contamination of reagents.
7. Remove the guide and the dynamic mask.
   - Remove the segment holder using its handle.
   - Remove the antisera segment from the holder and discard.

     WARNING : Segments with antisera have to be handled with care.
   - Residual reagent may remain in the wells after application. This should have no effect on test results.
8. Close the lid of the migration module.
9. Start the procedure immediately by pressing the green arrow "START" key on the left side of the keyboard.
   A message "[INCUBATION]" appears on the screen.

IMMUNOFIXATION - DESCRIPTION OF THE AUTOMATED STEPS

- Incubation at 20 °C for 5 minutes (controlled by Peltier effect).
- An audible beep signals that the migration module lid unlocks.
  The following message is displayed on the screen: "⇩ PAP." / "⇩ THICK FILTER PAPER".
  NOTE: The migration module lid remains locked during incubation.

IV. BLOTTING OF THE GEL

1. Open the migration module lid.
2. Apply a thick filter paper on the gel:
   line up the filter paper edge with the gel edge (incline it at a 45° angle) and ease it down onto the gel.
   IMPORTANT: Press firmly on the whole surface of the filter paper to ensure perfect adherence on the gel.
3. Close the lid of the migration module.
4. Start the procedure by pressing the "START" key (green arrow on the left side of the keyboard).

BLOTTING - DESCRIPTION OF THE AUTOMATED STEPS

- Blotting at 40 °C controlled by Peltier effect, for 3 minutes.
  The following message is displayed on the screen: "[BLOTTING]".
- An audible signal (beep) rings.
  The following message is displayed on the screen: "⇧ PAP." / "⇧ THICK FILTER PAPER".

V. DRYING OF THE GEL

1. Open the migration module lid.
2 Remove the filter paper and leave the gel in place.
3. Close the lid.
4. Start the procedure by pressing the "START" key (green arrow on the left side of the keyboard).

DRYING - DESCRIPTION OF THE AUTOMATED STEPS

- Drying at 50 °C controlled by Peltier effect, for 6 minutes.
- A beep signals that the cover unlocks. The plate temperature remains at 50 °C until the lid is opened. "MAINTAINED MIGR. TEMPERATURE" / "MAINTAINED TEMPERATURE" is displayed on the screen.

NOTE: The migration module lid remains locked during the drying step.

VI. GEL PROCESSING SET UP

1. Open the lid.
2. Remove the dried gel for further processing.
3. Open the gel holder. Lay it flat and position the dried gel (with gel side facing up) into the grooves of the two rods and close the holder. Make sure that the film is correctly positioned inside the holder (Fig. 13).
4. Place the gel holder into the Gel Processing / Staining Module.

   IMPORTANT: Before starting the gel processing / staining program, check the following:
   - the wash solution container contains at least 400 mL of wash solution ;
   - the staining container is filled with 300 mL of staining solution ;
   - the destaining container contains at least 1 liter of destaining solution ;
   - the waste container is empty.

   For reagent line connection: refer to the information displayed on the screen of the instrument (select key: REAGENT LINES).
   IMPORTANT: Do not forget to block up the unused lines.
5. Select "IF ACID VIOLET" or "IF AMIDO" staining program from the instrument menu and start the run by pressing the "START" key (green arrow on the right side of the keyboard).

   During staining, destaining and drying steps, the compartment remains locked.
   After cooling step, an audible beep signals that the compartment unlocks (the ventilation is maintained until the gel holder is removed).

NOTES:

- *Temperature of the migration plate keeps decreasing since the lid has been opened until it reaches 20 °C (in less than 5 minutes). Then a new migration run may start.*
- *Return the sample applicator and electrode carriers back in place.*
- *Wipe the temperature control plate with a soft wet tissue.*

VII.GEL PROCESSING COMPLETION

1. Remove the gel holder from the compartment, open it and remove the dried gel.
2. If needed, clean the back side (the plastic support side) of the dry film with a damp soft paper.

NOTE: The lengths of electrophoretic migrations may be slightly different with gels containing 2 or 3 analysis rows, without any adverse effects on performance.

QUALITY CONTROL

It is recommended to run an assayed control serum (such as IT / IF Control, SEBIA PN 4788) after each change of lot of a reagent.

RESULTS

Interpretation

1. *Serum*

Absence of monoclonal component

- A normal serum sample shows a light diffused staining of polyclonal immunoglobulins in all tracks.
- A hypergammaglobulinemia is characterized by a heavily stained, diffused gamma zone and absence of any restricted bands.

Presence of a monoclonal component

- The presence of a monoclonal protein (gammopathy) is characterized by a monoclonal band detected with one of the anti-heavy chain antisera (gamma, alpha or mu) and either with anti-kappa or anti-lambda light chain antiserum. The detected monoclonal band, typically sharp and demarcated in appearance, must be located at the same migration distance as the suspect monoclonal band seen in the reference track (ELP).
- Absence of reaction with any of the applied anti-heavy chain antisera and reaction with one of the light chain antisera might indicate :
  a) a very rare Ig D or Ig E gammopathy: confirm with anti-delta or anti-epsilon heavy chain antisera,
  b) a light chain gammopathy: confirm with antisera anti-kappa or anti-lambda free light chains.
- Failure to observe a positive reaction with any of the applied anti-light chain antisera, while an anti-heavy chain antiserum reacts, might indicate a very rare heavy chain gammopathy (gamma, alpha or mu).

Presence of two or more monoclonal components

In rare cases, several clones of B-cells proliferate as indicated by several monoclonal bands revealed by immunofixation:

- A biclonal gammopathy is characterized by the presence of two bands of heavy chain (identical or different) and two bands of light chains (identical or different).
- Polymerized immunoglobulins are characterized by several (usually two) bands of the same type of heavy chain and one of the same type of the light chain.
  To confirm the presence of a single monoclonal abnormality, it is necessary to depolymerize with beta-mercaptoethanol (BME or 2ME) and repeat immunofixation (see "Samples for analysis").
- An oligoclonal gammopathy is characterized by the presence of multiple, usually weak bands of one or more types of heavy chains and by one or two types of light chains.

Special cases

- When a monoclonal type band is observed on serum electrophoresis (ELP track) but fails to be confirmed by immunofixation, fibrinogen (plasma sample) should be the prime suspect.
- When a monoclonal type band is observed on all immunofixation tracks, cryoglobulin or polymerized Ig M should be suspected. Depolymerize with a reducing agent and repeat the procedure (see "Samples for analysis").
- For some Ig A gammopathies, the anti-light chain antiserum may present a faint affinity with the corresponding monoclonal immunoglobulin, and its detection is more difficult. In that case, it is recommended to test the sample with BENCE JONES immunofixation procedure where the antiserum reaction is amplified due to a longer incubation time (see the package insert of HYDRAGEL BENCE JONES kits for serum dilutions).
- With polyclonal background, it is recommended to use higher dilution of the sample for antisera tracks, and especially for Ig G track.
- HYDRAGEL 9 IF: For oligoclonal gammapothies, it is recommended to use HYDRAGEL 1, 2 or 4 IF immunofixation procedures to obtain a better resolution in gamma zone where elongated gammaglobulin zone allows multiple bands visualization.

2. *Urine*

Interpretation concepts similar to those shown for serum apply to urine immunofixation patterns.

- An intact monoclonal protein in urine is characterized by a monoclonal band detected with one of the anti-heavy chain antisera (gamma, alpha or mu) and with either anti-kappa or anti-lambda light chain (free & bound) antiserum. The detected monoclonal band, typically sharp and demarcated in appearance, must be located at the same migration distance as the suspect monoclonal band seen in the reference track (ELP).
- A monoclonal free light chain, Bence Jones protein (a tubular protein), is characterized by a monoclonal band detected with either anti-kappa or anti-lambda light chain (free & bound) antiserum. No positive reaction would be seen in any of the anti-heavy chain antisera (gamma, alpha or mu) tracks.

Interference and Limitations

See **SAMPLES FOR ANALYSIS**.

Fibrinogen or residual fibrin may lead to an artefact band on all tracks; its intensity, generally more important on the Ig A track, may vary according to the track.

The use of antisera other than those specific for the immunofixation procedure with the dynamic mask may affect the results.

Due to the resolution and sensitivity limits of zone electrophoresis, it is possible that some monoclonal components may not be detected with this method.

Troubleshooting

Call Technical Service of the supplier when the test fails to perform while the instruction for the preparation and storage of materials, and for the procedure were carefully followed.

Kit reagent Safety Data Sheets and information on waste product elimination are also available from the Technical Service of the supplier.

PERFORMANCE DATA

Reproducibility within gel and specificity

HYDRAGEL 4 IF procedure

Reproducibility within gel was demonstrated on three different pathological samples : two samples with one high level monoclonal component and one sample with two low levels monoclonal components. Each sample was run 4 times on 2 lots of HYDRAGEL IF 2/4 gels using the acid violet staining procedure.

HYDRAGEL 9 IF procedure

Reproducibility within gel was demonstrated on three different pathological samples each with one high level monoclonal component. Each sample was run 9 times on 2 lots of HYDRAGEL 9 IF gels using the acid violet staining procedure.

All repeats gave identical results within lot and lot-to-lot and as expected for the type of samples tested.

Reproducibility between gels and specificity

HYDRAGEL 4 IF procedure

Reproducibility between gels was demonstrated on four different pathological samples with one or many monoclonal components.
These samples were run on 10 HYDRAGEL IF 2/4 gels from 3 different lots, using the acid violet staining procedure.

HYDRAGEL 9 IF procedure

Reproducibility between gels was demonstrated on nine different pathological samples : two samples with one high level monoclonal component, one sample with two low levels monoclonal components, and one sample with one high and one low level monoclonal component.
These samples were run on 10 HYDRAGEL 9 IF gels from 3 different lots, using the acid violet staining procedure.

All repeats gave identical results gel to gel and as expected for the type of samples tested.

Accuracy

HYDRAGEL 4 IF procedure with acid violet staining

Seventy three (73) different pathological serum samples, eleven (11) normal sera and twelve (12) concentrated urines were run using the HYDRAGEL IF 2/4 gels and another commercially available agarose gel immunofixation system.

HYDRAGEL 4 IF procedure with amidoblack staining

Twenty eight (28) different pathological serum samples and four (4) normal sera were run using the HYDRAGEL IF 2/4 gels and another commercially available agarose gel immunofixation system.

HYDRAGEL 9 IF procedure with acid violet staining

Ninety five (95) different pathological serum samples, four (4) normal sera and twelve (12) concentrated urines were run using the HYDRAGEL 9 IF gels and another commercially available agarose gel immunofixation system.

In all cases, the results obtained by the SEBIA tests and the comparable commercially available procedure were identical.

Sensitivity
Serial dilutions were prepared with 4 pathological serum samples all exhibiting monoclonal components and analyzed with HYDRAGEL 4 IF procedure. The acid violet and the amidoblack staining procedures were both tested.

The results are summarized below.

	MONOCLONAL COMPONENT		DETECTION LIMIT (mg/dL)	
SAMPLE No	**TYPE**	**CONC. (g/dL)**	**HYDRAGEL 4 IF ACID VIOLET**	**HYDRAGEL 4 IF AMIDOBLACK**
1	gamma kappa	1.09	12 12	12 12
2	gamma lambda	0.78	12 12	/ /
3	alpha lambda	0.58	12 12	25 25
4	mu kappa	0.34	12 12	12 12

Depending on the migration position of the monoclonal component, the level of polyclonal background in the gamma zone and the staining procedure, the detection limit may vary from 12 to 25 mg/dL.

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Pour des informations complémentaires sur l'interprétation des profils obtenus par immunofixation, voir :
For additional information on interpretation of immunofixation patterns refer to:

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SCHÉMAS / FIGURES

Figure 1

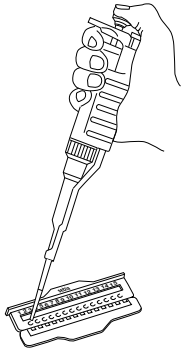
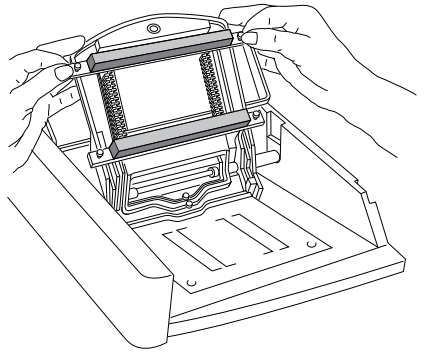


Figure 2



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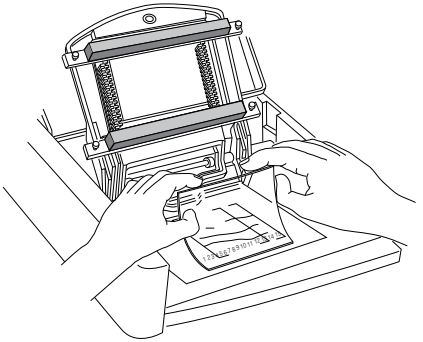

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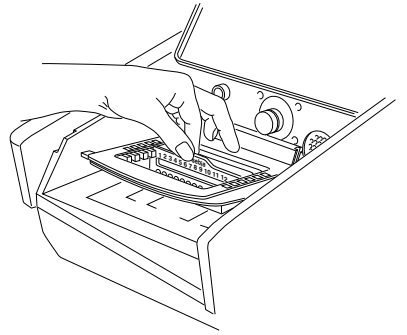
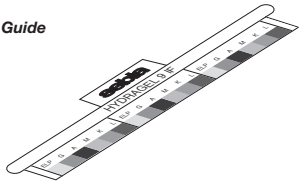


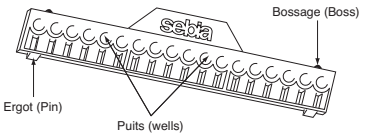
SCHÉMAS / FIGURES

Figure 5

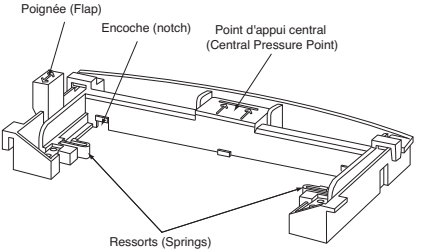
Repère couleurs
Colored Reference Guide



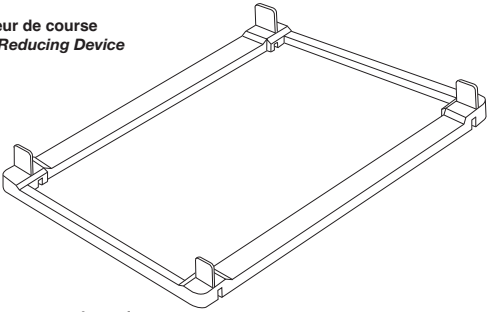
Barrette antisérums
Antisera Segment



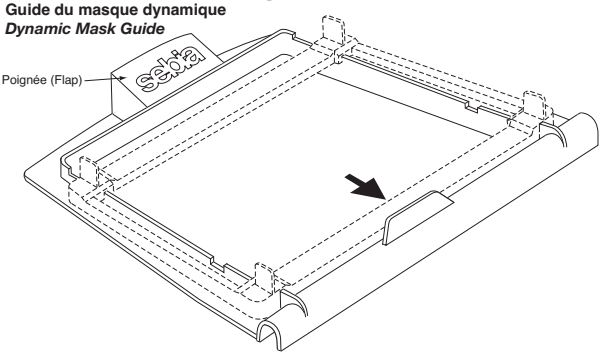
Support barrette
Segment Holder



Réducteur de course
Length Reducing Device

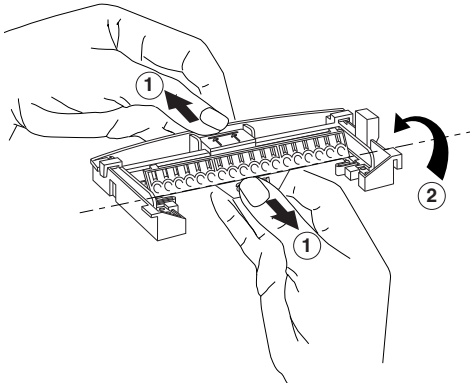


Guide du masque dynamique
Dynamic Mask Guide



SCHÉMAS / FIGURES

Figure 6



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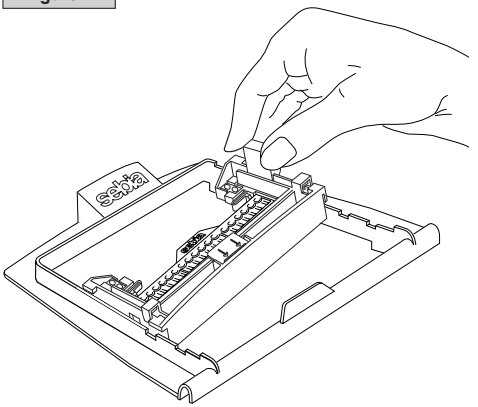

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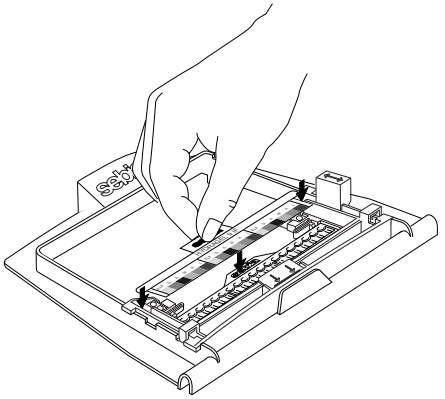

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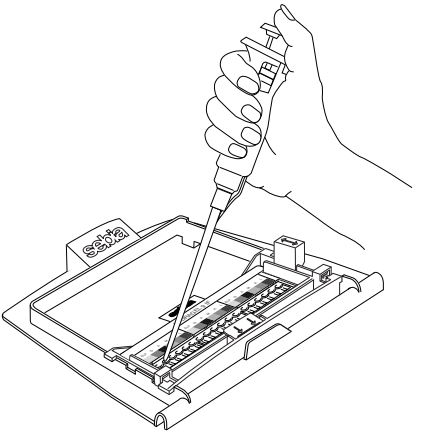

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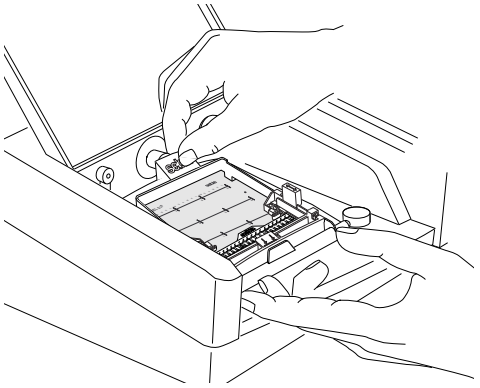

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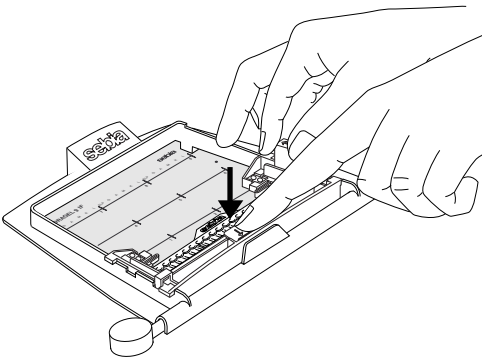

SCHÉMAS / FIGURES

Figure 12

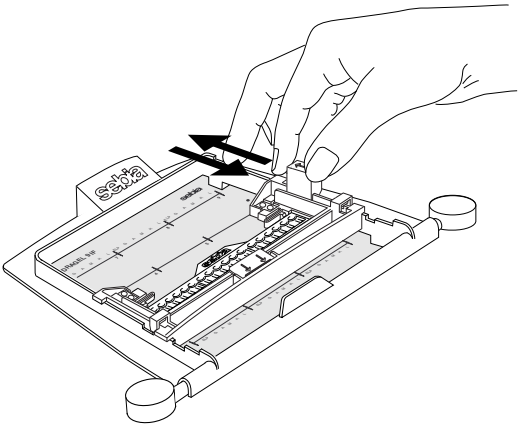

Figure 13