

# LIVER PROFILE DOT KIT

## M2 PDH – LKM1 – LC1 – SLA – F actin

Order Code: AD-LIVERD  
24 Tests - For in vitro use only

### 1. INTENDED USE

Alphadia Liver Profile DOT Kit is an Immunodot kit intended for the detection of IgG autoantibodies against M2 (Pyruvate Dehydrogenase), LKM1 (P450 2D6), LC1 (Formiminotransferase Cyclodeaminase), SLA (soluble liver antigen) and F-actin in human serum.

### 2. INTRODUCTION

Primary Biliary Cirrhosis (PBC) and Autoimmune Hepatitis (AIH) are chronic liver diseases with distinct clinical and immunological features. The diagnosis is based on a combination of clinical, biochemical, histological and serological findings.

Apart from testing for markers of viral hepatitis, particularly the hepatitis B and C viruses (in order to exclude these as a cause of the liver disease), the serology is mainly concerned with screening for autoantibodies.

**M2** antibodies belong to the group of antimitochondrial antibodies (AMA) and are strongly associated with PBC. At least nine distinct AMA have been identified, which have been classified as M1-M9 according to their antigen specificity and disease association. Of these, only the M2 subtype (which reacts with epitopes in the E2 components of the pyruvate dehydrogenase complex) seems to approach absolute specificity for PBC. Indeed about 95 % of patients with PBC have M2 autoantibodies and, conversely, about 90 % of asymptomatic individuals who are found to be M2-positive on routine screening can be shown to have underlying PBC on further investigations.

**LKM1 (Liver/Kidney Microsome)** antibodies react with the microsomal cytochrome isoform P450 IID6 and are the most prevalent markers for type II AIH. They are considered specific for the disease, although low titres of antibodies have been reported in about 5 % of patients with chronic hepatitis C.

**LC1 (Liver Cytosol)** antibodies react with a soluble cytosol enzyme (Formiminotransferase Cyclodeaminase) and occur in about 30 % of patients with type II AIH. Although they are frequently associated with LKM1 antibodies, they are the sole liver-related circulating autoantibodies in about 10 % of cases. Moreover anti-LC1 antibodies are clinically associated in most cases with greater disease activity and younger age.

**SLA (Soluble Liver Antigen)** antibodies seem to have a high positive predictive value for type I AIH. Although they are relatively rare (<30%), they occur in a significant number of patients who are seronegative at presentation for other autoantibodies and therefore may be useful diagnostically.

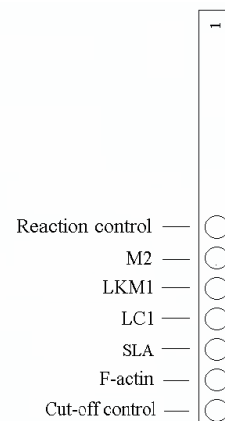
**F-actin** antibodies are the main component of the broad family of Smooth Muscle antibodies (SMA). They bind to the F-actin component in the cytoskeleton of the cells and are highly predictive for type I AIH, with a sensitivity and specificity of about 80 % and 90 %, respectively.

**Alphadia Liver Profile DOT Kit combines easy handling and cost effectiveness for the reliable detection of the most relevant antibodies in autoimmune hepatic disorders in a single test.**

### 3. PRINCIPLE OF THE TEST

The test is based on the principle of an Enzyme immunoassay.

The test strip is composed of a membrane fixed on plastic support. During test procedure, the strips are incubated with diluted patients sera. Human antibodies, if present, bind to the corresponding specific antigen(s) on the membrane. Unbound or excess antibodies are removed by washing and AP-conjugated goat antibodies against human IgG are added to the strips. This enzyme conjugate binds to the antigen-antibody complexes. After a second washing step to remove excess conjugate, substrate solution is added. Enzyme activity, if present, leads to the development of purple dots on the membrane pads. The intensity of the coloration is directly proportional to the amount of antibody present in the sample.



#### 4. KIT CONTENTS

**Abbreviations :**

TBS = Tris Buffer Saline ; BSA = Bovine Serum Albumin ; MIT = MethylIsoThiazolone ;  
AP = Alkaline Phosphatase ; NBT = NitroBlue Tetrazolium ; BCIP = Bromo-Chloro-Indolyl-Phosphate.

TO BE DILUTED :

**(10 X) Wash buffer :**        **1 x 50 ml** (colourless)  
*Contains : TBS, Tween ; Preservative : MIT*

READY TO USE :

**Dot strips :**                    **24 units**  
*(7 Dots each: 1 reaction control, 5 antigens, 1 cut-off control)*

**Diluent buffer :**                **1 x 40 ml** (yellow)  
*Contains : TBS, BSA, Tween ; Preservative : MIT*

**Conjugate :**                      **1 x 40 ml** (red)  
*Contains : AP-conjugated goat anti-human IgG ; Preservative : MIT*

**Substrate :**                      **1 x 40 ml** (brown bottle, pale yellow solution)  
*Contains : NBT/BCIP ; Preservative : 0.05 % NaN<sub>3</sub> (sodium azide)*

**Incubation trays:**              **3 units**  
*(with 8 wells for incubation)*

#### 5. MATERIAL REQUIRED BUT NOT PROVIDED

Rocking or shaking platform / Micropipettes / Timer / Graduated cylinder / Distilled or deionised water / Tweezers / Absorbent and/or filter paper

#### 6. STORAGE

The reconstituted Wash Solution is stable for at least one month at 2-8°C. Reagents and strips can be stored at 2-8°C until the expiry date indicated on each vial or tube. Place unused strips back into the provided tube, seal it and store at 2-8°C. Chromogen/Substrate (NBT/BCIP) shall be stored at 2-8°C.

#### 7. PRECAUTIONS

All reagents are for in vitro diagnostic use only. The kit contains potentially hazardous components thus avoid contact with skin, eyes or mucosae. Patient samples shall be handled as potentially infectious. Do not substitute reagents or mix strips with different batch numbers this may lead to variations in the results. Avoid touching strips with fingers. Use tweezers or wear laboratory gloves. Allow reagents and strips to equilibrate at room temperature before use. Strictly observe incubation times. Handle Chromogen Substrate (NBT/BCIP) with care in order to avoid any contamination with Alkaline Phosphatase.

#### 8. SAMPLE COLLECTION, HANDLING AND STORAGE

Samples should be preferentially fresh-collected ones. Sera with debris should be low speed centrifuged. Blood samples should be collected in dry tubes or in tubes containing EDTA or heparin. After separation serum samples shall be used immediately or aliquoted and stored at 2-8°C for some days or frozen at -20°C for longer periods. Avoid repeated freezing thawing cycles.

## 9. ASSAY PROCEDURE

### BASIC HANDLING AND TIPS :

The dots are precoloured blue on the strips, ensuring that all antigens have been dotted correctly onto the membrane. This blue coloration disappears during the first step of the procedure. During incubation with the sample a faint pink background coloration appears on the membrane and disappears upon drying at the end of the procedure.

During the procedure, agitation of the incubation tray is necessary to ensure efficient circulation of fluids over the membrane. Any kind of shaking or rocking platform may be used if the amplitude of the movement does not cause spilling out of solutions and cross-contamination between the wells. Agitation speeds ranging from 10 to 60 rpm are suitable, with 20–30 rpm being preferred.

After each filling of the wells with solution, agitate manually the incubation tray to ensure that the strips are completely immersed and to remove air bubbles which may be trapped under the strip. Alternatively, floating strips may be forced into the solution by pushing down (with tweezers or pipette tip) on the upper part of the strip (plastic label zone).

Avoid touching the membrane zone of the strip with fingers, tweezers or pipette tips. Always use the plastic label zone for handling or manipulation. The whole procedure has to be run at room temperature

### 9.1 Reagents preparation

1. Allow all components to equilibrate at room temperature before use.
2. Dilute the concentrated Wash Buffer 10x with distilled water.

*Prepare 15 ml diluted Wash buffer per strip tested*

*Example: 1,5 ml concentrated wash buffer + 13,5 ml distilled water for one strip.*

### 9.2 TECHNIQUE

1. Place one strip per patient into the wells, blue dots facing up.
2. Add 2 ml Wash Buffer to each well. Incubate (shake) for 10 min.  
*Upon correct incubation the blue coloration of the dots completely disappears.  
If not prolong the procedure until the colour of the dots fades completely.*
3. Discard solution from the wells.  
*Remove liquid by slowly inverting the plate. The strips will adhere to the bottom of the wells. Dry the edges of the tray with absorbent paper.*
4. Add 1,5 ml Sample Diluent to the wells.
5. Add 10µl patient sample to the wells. Incubate (shake) for 30 min.  
*Avoid touching the membrane with the pipette tip. Preferentially dispense the sample in the solution over the upper part of the strip (plastic label zone).  
Note : Steps 4 and 5 can be combined by pre-diluting the sample in a glass or plastic tube (1,5 ml diluent + 10µl patient sample -> Mix -> Add to the well)*
6. Wash 3 x 3 minutes with 1,5 ml Wash Buffer (shake).  
*Remove liquid from the wells by slowly inverting the plate. The strips will adhere to the bottom of the wells. Dry the edges of the tray with absorbent paper.*
7. Add 1,5 ml Conjugate to each well. Incubate (shake) for 30 min.
8. Wash 3 x 3 min. with 1,5 ml Wash Buffer (see 6.)
9. Add 1,5 ml Substrate to the wells. Incubate (shake) for 10 min.
10. Wash 1 x 3 min. with 1,5 ml Wash solution to stop the reaction.
11. Collect the strips from the wells and allow them to dry on absorbent paper.

## 10. RESULTS INTERPRETATION

### 10.1 Interpretation

1. Peel off the cover of the adhesive on the back side of each strip and attach strips dots face up onto the marked fields of the interpretation sheet provided with the kit. This will indicate the respective positions of the different controls and antigens on the membrane.
2. Check the first upper Dot (reaction control): it must be positive for all patients.  
*Only a clearly coloured positive Reaction Control Dot ensures your results are valid and operation was correct and/or kit components were not degraded.*
3. Compare the specific antigen Dots to the cut-off Dot which always is the last in order.  
*The colour intensity of the Antigen Dots is directly proportional to the titer of the specific antibody in the patients sample.*  
***Under optimum conditions and if the sample is free of interfering substances the cut-off dot may be even close to uncoloured. In contrast a highly coloured cut-off Dot indicates a high rate of unspecific binding in the sample.***

**POSITIVE RESULT:** A sample is positive for a specific antibody if the colour intensity of the corresponding Antigen Dot is higher than the intensity of the cut-off Dot.

**NEGATIVE RESULT:** A sample is negative for a specific antibody if the colour intensity of corresponding Antigen Dot is lower or equal than the intensity of the cut-off Dot.

## 11. TEST LIMITATIONS

Alphadia Liver Profile DOT Kit is intended for the detection of IgG autoantibodies to M2, LKM1, LC1, SLA and F-actin. It serves as an aid for the differential diagnosis of PBC and AIH Type 1 and Type 2. Results have to be interpreted in the light of the clinical examination and case history.

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