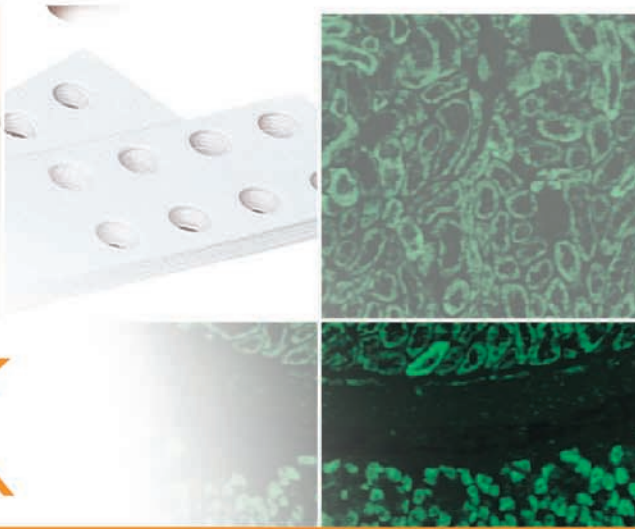




Bio-Rad Laboratories
The Clear Choice in Autoimmune Testing



MK/MSK

Autoimmune IFA

A U T O I M M U N E T E S T I N G

Kallestad™ The name you know; the quality you trust

Bio-Rad offers highly sensitive rodent tissue sections that can be used to detect Anti-Mitochondrial Antibodies (AMA), Anti-Smooth Muscle Antibodies (ASMA), Anti-Parietal Cell Antibodies (APCA) and other organ-specific autoantibodies. The use of this tissue section minimizes interference from blood group isoantibodies while providing easily discernible fluorescent patterns.



The Bio-Rad Kallestad™ Mouse Kidney and Mouse Stomach/Kidney, Kits are indirect fluorescent antibody assays for the detection and semi-quantitation of human autoantibodies.

Product Highlights

- Universal procedure (HEp-2, MK, MSK and *Crithidia lucillae*)
- 8-well slide configuration
- Universal polyclonal conjugate
- Standardized and rigidly controlled manufacturing procedures
- Ready-to-use controls

Clinical Utility

The detection and semi-quantitation of autoantibodies aid in the diagnosis of autoimmune diseases.¹ Laboratories have used indirect fluorescent antibody (IFA) procedures to detect autoantibodies since 1957. In these procedures, a fluorescent antibody serves as a marker for an antigen-antibody binding reaction which occurs on the surface of the substrate. Among the substrates commonly used in the IFA procedure are mouse kidney and stomach, human epithelial (HEp-2) cells, and *Crithidia lucillae*, a hemoflagellate. Observation of a specific pattern of fluorescence on the substrate indicates the presence of autoantibodies in the patient's serum.

A high AMA titer supports the diagnosis of primary biliary cirrhosis. Low titers of AMA may be detected in other liver disorders which include chronic active hepatitis and cryptogenic cirrhosis.^{1,2} ASMA is present in high titers in the serum of 70% of patients with chronic active hepatitis. In addition, 50% of these patients are positive for ANA, while 25% demonstrate low AMA titers. Low ASMA titers may be present in viral infections, malignancies, and good health. ASMA usually does not appear in SLE.^{1,3,4}


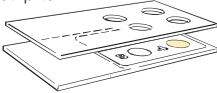

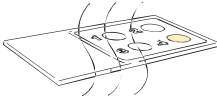
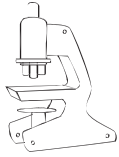
APCA occurs in the serum of 90% of patients with autoimmune pernicious anemia. With other clinical and laboratory data, a positive APCA result helps to distinguish autoimmune pernicious anemia from other megaloblastic anemias.





Kallestad™ Mouse Kidney and Mouse Stomach/Kidney

Autoimmune IFA Procedure – MK/MSK

- 1 Reconstitute PBS with distilled water.
- 2 Prepare screening dilutions of the test sera as specified in the instruction manual.
- 3 Remove appropriate number of slides from freezer or refrigerator and equilibrate to room temperature in foil bags (20 minutes). Bring all reagents to room temperature.
- 4 Remove slide from foil bag and place in moisture chamber. Immediately add 25 µL of controls or diluted test sera to appropriate wells.
 
- 5 Cover moisture chamber/slide holder. Incubate substrate slides at room temperature for 20 minutes.
- 6 Gently rinse each slide with PBS buffer and remove excess buffer.
- 7 Wash slides for 10 minutes in a staining dish filled with PBS. Gently agitate several times or use a magnetic stirrer during the wash period.
- 8 Remove each slide or slide holder from staining dish and blot excess PBS from around wells using blotting strips. Do not touch strip to substrate.
 
- 9 Place slide(s) in moisture chamber and immediately dispense 25 µL FITC Conjugate to each well.
 
- 10 Cover moisture chamber/slide holder. Incubate 20 minutes at room temperature.
- 11 Rinse each slide briefly with a stream of PBS. Wash slides for 10 minutes in a staining dish filled with PBS or perform an optional counterstaining procedure (using Evans' Blue Counterstain) as specified in the instruction manual.
 
- 12 Remove each slide or slide holder from PBS and drain briefly on a paper towel. Apply 4 ± 1 drops mounting media per slide, making sure to cover all wells and add cover slip.
- 13 Examine the reactions on the slide under a fluorescent microscope.
 

Result	Interpretation
Negative	No specific pattern of fluorescence on the substrate, such as ANA (Homogeneous – diffuse, Rim – Peripheral, Speckled, Nucleolar), AMA (Mitochondrial), ASMA (Smooth Muscle), APCA (Parietal Cell), Brush Border or Reticulin or < +1 fluorescence
Positive	When substrate shows a specific pattern of fluorescence with ≥ +1 fluorescence

REFERENCES

1. Peter, J. B., Dawkins, R.L. September-October 1979. Evaluating autoimmune diseases. *Diagnostic Medicine*, 68-76.
2. Doniach, D., Roitt, I.M., Walker, J.G., Sherlock, S., 1966. Tissue antibodies in primary biliary cirrhosis, active chronic (lupoid) hepatitis, cryptogenic cirrhosis and other liver diseases and their clinical implications. *Clinical Experimental Immunology*, 1: 237-262.
3. Toh, B.H. 1979. Smooth muscle autoantibodies and autoantigens. *Clinical Experimental Immunology*, 38: 621-628.
4. Lange, A., et al. 1978. Diagnostic specificity of autoantibodies. *Archivum Immunologiae et Therapiae Experimentalis*, 26: 881-885.

Ordering Information

Catalog No. Description

Complete Kits

30443	Kallestad™ Mouse Stomach/Kidney Kit (6 x 8w slides)48 tests
30445	Kallestad™ Mouse Kidney Kit (6 x 8w slides)48 tests
30447	Kallestad™ Mouse Kidney Kit (24 x 8w slides)192 tests

Components and Accessories

30441	Kallestad™ Mouse Stomach/Kidney Slide 8 wells
30434	Kallestad™ Mouse Kidney Slide 8 wells
30446	Universal FITC Conjugate 2.5 mL
30480	Universal FITC Conjugate 100 mL
30403	Mounting Medium 2.5 mL
31098	Phosphate Buffered Saline (PBS) (1 liter reconstituted) 11.34 grams
30431	Evans' Blue Counterstain 2.5 mL

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For further information, please contact the Bio-Rad office nearest you or visit our website at www.bio-rad.com.

Clinical Diagnostics Group

Website www.bio-rad.com U.S. 1-800-2-BIO-RAD Australia 61-2-9914-2800 Austria 43-1-877-8901 Belgium 32-9-385-5511 Brazil 5521-2507-6191 Canada 1-514-334-4372 Czech Republic 420-2-41430532 China 86-21-63052255 Denmark 45-4452-1000 Finland 358-9-804-22-00 France 33-1-4795-6000 Germany 49-89-31884-0 Hong Kong 852-2789-3300 India 91-124-6398112 Israel 972-3-9514127 Italy 39-02-216091 Japan 81-3-5811-6290 Korea 82-2-3473-4460 Latin America 305-894-5950 Mexico 5255-5534-2552 The Netherlands 31-318-540666 New Zealand 64-9-415-2280 Norway 47-23-38-41-30 Poland 48-22-331-99-99 Portugal 351-21-472-7700 Russia 7-095-721-14-00 Singapore 65-6415-3188 South Africa 27-11-442-85-08 Spain 34-91-590-5200 Sweden 46-8-555-127-00 Switzerland 41-61-717-95-55 Thailand 662-651-8311 United Kingdom 44-208-328-2000