Anti-Mitochondrial Antibody (AMA) Reference Material for ICAP Pattern AC-21


**Intended Use:** For *in vitro* immunodiagnostic use as a human reference standard having high levels of antibodies to components of the mitochondria and be used to confirm the specificity of local standards.

**Description of Reference Material:** Defibrinated human plasma from a single donor was donated by Plasma Services Group Inc. (PSG) in Huntingdon Valley, PA. Volumes of 200 µL were dispensed into borosilicate glass vials, freeze-dried, and sealed with butyl rubber stoppers while still under reduced pressure. Vials are stored at PSG at -20°C and delivered directly to users from PSG.

**Reconstitution and Storage:** Store the freeze-dried material at -20°C until use. To reconstitute, the contents should first be shaken to the bottom by gently tapping the upper end or by gently tapping the bottom of the vial on the lab bench. Before the stopper is removed, the vacuum should be broken by insertion of a hypodermic needle through the rubber stopper. Precisely 200 µL of distilled water should then be added, and the vial re-stoppered. The freeze-dried powder should dissolve readily with gentle swirling (avoid foam). Allow to stand for at least 1 h before use and store at 4°C if planning to use within 24 h. Otherwise, the reconstituted standard can be aliquoted and stored at -20°C or lower for later use.

**Caution:** This material was tested and found negative or non-reactive for STS, HBsAg, HIV 1 Ag (or HIV PCR (NAT)), HCV antibody, HCV PCR (NAT) and HIV1/2 antibody as required at the time of bleeding using FDA-licensed test kits. Since no method can offer complete assurance that these or other infectious agents are absent, this reference material should be handled as if capable of transmitting infection.

**Anti-Mitochondrial Antibody Content:** This human reference material contains antibodies to components of the mitochondria, which appear as a coarse granular filamentous or reticular staining indirect immunofluorescence (IF) staining pattern in the cytoplasm of HEp-2 cells (AC-21) and cryopreserved sections of rodent kidney. This reference material reacts strongly with major antigens of the pyruvate dehydrogenase complex (PDC), including dihydrolipoyl transacetylase (E2), dihydrolipoamide dehydrogenase (E3), E3-binding protein (E3BP), and PDC subunits E1α and E1β, which are recognized targets of antibodies from patients with primary biliary cholangitis. Eight international laboratories confirmed the specificity of this reference material. Methods used include immunoprecipitation (IP), western blotting (WB), line immunoassay (LIA), chemiluminescence (CIA), and addressable laser bead immunoassay (ALBIA).

**TECHNICAL DETAILS & APPLICATIONS**

**Purification Method:** Not purified, defibrinated human plasma

**Autoantigens identified:** dihydrolipoyl transacetylase (PDC-E2, encoded by DLAT, Gene ID 1737), dihydrolipoamide dehydrogenase (PDC-E3, encoded by DLD, Gene ID 1738), E3-binding protein (E3BP, encoded by PDHX, Gene ID 8050), pyruvate dehydrogenase E1α (PDHA1, Gene ID 5160), pyruvate dehydrogenase E1β (PDHB, Gene ID 5162)

**Calculated MWs:**

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<tbody>
<tr>
<td>E2</td>
<td>69 kD (647 aa)</td>
<td>74 kD</td>
</tr>
<tr>
<td>E3</td>
<td>54 kD (509 aa)</td>
<td>58 kD</td>
</tr>
<tr>
<td>E3BP</td>
<td>54 kD (501 aa)</td>
<td>54 kD</td>
</tr>
<tr>
<td>E1α</td>
<td>43 kD (390 aa)</td>
<td>41 kD</td>
</tr>
<tr>
<td>E1β</td>
<td>39 kD (359 aa)</td>
<td>34 kD</td>
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**Observed MWs in IP and WB:**

**Storage:** After reconstitution in 200 µL water as described above, store at -20°C or lower.

**Storage Buffer:** The reference material contains 4% Na Citrate as an anticoagulant. No preservatives have been added.

**Tested Applications:** IF, IP, WB, LIA, ALBIA, and CIA.

**Known Species Specificity:** Human
**Tested Species:** Human  

**Recommended Dilutions:**  
- IF on HEp-2: Starting dilution 1:80, positive to at least 1:1280  
- IF on rodent kidney/liver/stomach: Starting dilution 1:40, positive to at least 1:1280  
- IP: undiluted (use 8 µL reference material)  
- WB: 1:3000 – 1:5000  
- LIA, ALBIA, other PDC assays: follow manufacturer’s protocol  

**Notes:** This reference material yields a typical cytoplasmic coarse granular reticular pattern (AC-21) by IIF on HEp-2 cells, with no additional staining. In the IIF on cryopreserved rodent kidney/liver/stomach sections, there is a characteristic coarse granular staining in the cytoplasm of hepatocytes and kidney tubular cells. The staining of tubular cells is more prominent and consistent at the distal tubes and there may be some heterogeneity of staining in proximal tubules.

**VALIDATION DATA**

![Images with dilutions from 1:40 to 1:1280 are shown, labeled by Fcγ fragment specific goat anti-human IgG conjugated to Alexa Fluor 488. The reference material yields a cytoplasmic coarse granular reticular/AMA pattern at all dilutions. No additional staining is observed.](image)

IIF of the reference material on HEp-2 substrate from Inova Diagnostics. Images with dilutions from 1:40 to 1:1280 are shown, labeled by Fcγ fragment specific goat anti-human IgG conjugated to Alexa Fluor 488. The reference material yields a cytoplasmic coarse granular reticular/AMA pattern at all dilutions. No additional staining is observed.

**REFERENCES**


