

Anti-DFS70/LEDGF-p75 (anti-DFS70) Reference Material for ICAP Pattern AC-2

Product Package Insert

Catalogue IS2726

Lot 18296

DOM: April 2018

Intended Use: For *in vitro* immunodiagnostic use as a human reference standard having high levels of antibodies DFS70/LEDGF-p75 (anti-DFS70) to be used to confirm the specificity of local standards.

Description of Reference Material: Human serum from 778 donors was collected at Fleury Medical Laboratory in São Paulo, SP, Brazil. After confirmation of mono-specific anti-DFS70 reactivity, the samples were consolidated in a single pool (designated Megapool). Volumes of 200 µL were dispensed into borosilicate vaccine vials, freeze-dried, and sealed with butyl rubber stoppers while still under reduced pressure. Vials are stored at Plasma Services Group Inc. (PSG) in Huntingdon Valley, PA, USA, 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 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-DFS70 Antibody Content: This human Reference Material contains antibodies to the 70/75kDa DFS70 antigen, also referred to as lens epithelium derived growth factor (LEDGF-p75) and transcription coactivator p75 (TCp75). This ubiquitous protein in mammalian cells is encoded by the gene PSIP1 (PC4 and SFRS1 interacting protein 1)^{reviewed in 1}. DFS70 that has a peculiar distribution across the interphase nucleoplasm and is enriched at the chromatin metaphase plate in mitosis². The present human reference material was extensively characterized as mono-specific anti-DFS70 reactivity. Eight international laboratories confirmed the specificity of this Reference Material³.

TECHNICAL DETAILS & APPLICATIONS

Purification Method: Not purified, human whole serum.

Autoantigen identified: DFS70 antigen, also referred to as lens epithelium derived growth factor (LEDGF-p75) and transcription coactivator p75 (TCp75). (PSIP1, NCBI Gene ID 11168; chromosome 9)

Calculated MW of DFS70: 60 kD (530 aa)

Observed MW of DFS70: 70-75 kD

Storage: After reconstitution in 200 µL water as described above, store at -20°C or lower. The reference material is stable at -20°C.

Storage Buffer: No preservatives have been added.

Tested Applications: Indirect immunofluorescence (IF), immunoprecipitation (IP), western blotting, enzyme-linked immunosorbent assay (ELISA), chemiluminescence immunoassay (CLIA), addressable laser bead immunoassay (ALBIA)

Known Species Specificity: Human

Tested Species: Human

Recommended Dilutions:

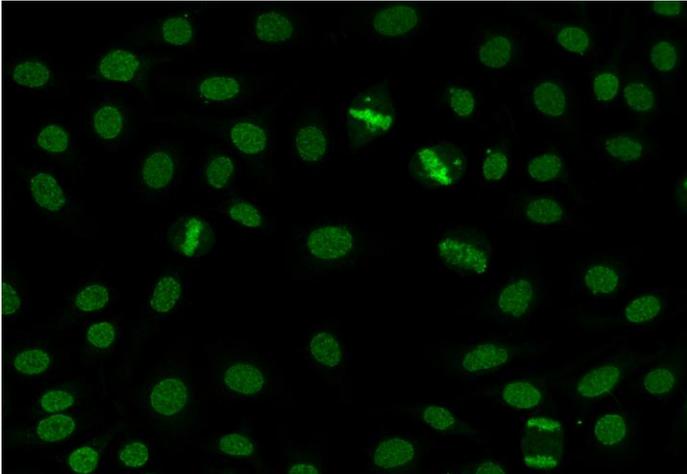
IF on HEp-2: Starting dilution 1:80, positive to at least 1:1280

IP: 10 μ L

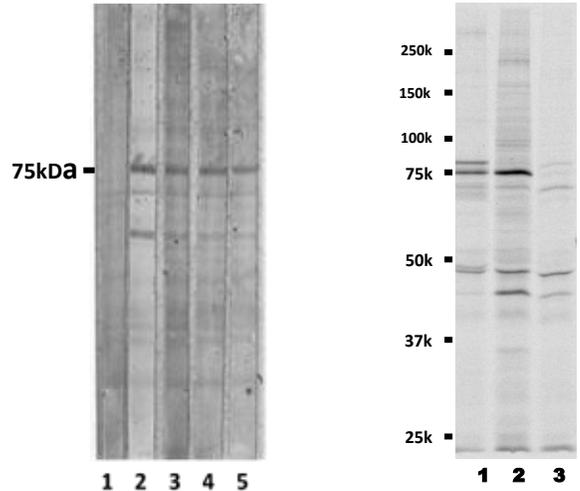
Notes:

To date, eight HEp-2 slide brands have been tested with this reference material and all yielded the expected AC-2 pattern.

VALIDATION DATA



Indirect immunofluorescence assays with reconstituted anti-DFS70 reference material using HEp-2 substrate from MBL/Bion. Dilution 1:640



(Left) Western blot of whole MOLT-4 lysate probed with normal human serum (1); reference anti-DFS70 serum provided by Carlos Casiano, Loma Linda University, California (2); anti-DFS70 reference material before lyophilization (3) and reconstituted after lyophilization (4 and 5).

(Right) total HeLa cell extract immunoprecipitated by human serum reactive with anti-DFS70 serum confirmed by ELISA (1), reconstituted anti-DFS70 reference material (2), and normal human serum (3).

REFERENCES

1. Ochs RL, Mahler M, Basu A, Rios-Colon L, Sanchez TW, Andrade LE, Fritzler MJ, Casiano CA: The significance of autoantibodies to DFS70/LEDGFp75 in health and disease: integrating basic science with clinical understanding. *Clin Exp Med.* 2016 16:273-93.
2. Mariz HA, Sato EI, Barbosa SH, Rodrigues SH, Dellavance A, Andrade LE: ANA-HEp-2 pattern is a critical parameter for discriminating ANA-positive healthy individuals and patients with autoimmune rheumatic diseases. *Arthritis Rheum.* 2011 63: 191-200.
3. Dellavance A, Baldo DC, Zheng B, Mora RA, Fritzler MJ, Hiepe F, Rönnelid J, Satoh M, Garcia-De La Torre I, Wener MH, Chan EKL, Andrade LEC: Establishment of an international autoantibody reference standard for human anti-DFS70 antibodies: proof-of-concept study for a novel Megapool strategy by pooling individual specific sera. *Clin Chem Lab Med.* 2019: doi: 10.1515/cclm-2019-0087