PCNA ANTIGEN

AROTEC_PCNA_Product_Info.pdf Version/Date: A/14.08.19

ATP03-02 PCNA antigen 0.20 mg
ATP03-10 PCNA antigen 1.0 mg

Description of the Product

Purified recombinant full-length human sequence protein with hexa-Histidine tag. After coating onto ELISA plates the product will bind autoantibodies to PCNA.

Purity: The PCNA autoantigen (34 kDa) is more than 90% pure, as assessed by SDS-polyacrylamide gel electrophoresis.

Storage: Store at -65°C or below (long term). Avoid repeated freezing and thawing. Mix thoroughly before use.

Clinical and Biochemical Data

Autoantibodies to proliferating cell nuclear antigen (PCNA) in the serum of patients with systemic lupus erythematosus (SLE) were first described in 1978[1,2]. Although anti-PCNA antibodies were initially believed to be specific for SLE[3,4], they have now been described in patients with Sjoegren's syndrome[5,6] as well as in a range of other clinical conditions such as hepatitis B[7,8], hepatitis C[7], and malignant disease[9,10]. Anti-PCNA antibodies in patients with SLE have been reported to correlate with a greater incidence of glomerulonephritis[11].

PCNA is a member of the DNA sliding clamp (β clamp) family and has an essential role in DNA synthesis[12]. The protein exists as a homotrimer[13,14] and acts by encircling DNA to serve as a scaffold on which DNA polymerases are tethered. PCNA is also known to interact with a number of other proteins[15] that are involved in DNA replication, repair or methylation or in cell cycle regulation. PCNA deletion mutant studies suggest that autoantibodies in SLE sera are very heterogeneous and can react with a range of different epitopes[16], whereas autoantibodies present in patients with hepatitis B recognise preferentially the C-terminal of PCNA[8]. Sulfhydryl groups present in PCNA also appear to be essential for autoantibody binding[17].

Recombinant full-length human-sequence protein expressed and purified from Sf21 insect cells constitutes AROTEC's PCNA antigen. The use of recombinant PCNA antigen for the detection human autoantibodies has been described elsewhere[6].

Methodology

The following is an ELISA procedure which can be used to detect anti-PCNA autoantibodies in human serum using the ATP03 purified PCNA autoantigen:

1. Dilute the purified antigen to 0.2-1.0 µg/ml in 10 mM Tris-HCl, pH 7.4; 0.15 M NaCl
2. Coat ELISA plates with 100 µl of diluted antigen per well. Cover and incubate 24 hours at +4°C.
3. Empty the plates and remove excess liquid by tapping on a paper towel.
4. Block excess protein binding sites by adding 200 µl PBS containing 1% BSA per well. Cover and incubate at +4°C overnight.
5. Empty plates and apply 100 µl of serum samples diluted 1:100 in PBS / 1% BSA / 1% casein / 0.1% Tween® 20. Incubate at room temperature for 1 hour.
6. Empty plates and add 200 µl PBS / 0.1% Tween® 20 per well. Incubate 5 minutes then empty plates. Repeat this step twice.
7. Apply 100 µl anti-human IgG-enzyme conjugate (horseradish peroxidase or alkaline phosphatase) diluted in PBS / 1% BSA / 1% casein / 0.1% Tween® 20 per well and incubate for 1 hour.
8. Repeat step 6.
9. Add enzyme substrate and stop the reaction when appropriate.
10. Read absorbance in an ELISA spectrophotometer.

References

1. Miyachi, K. et al. (1978) J. Immunol. **121**, 2228
2. Takasaki, Y. et al. (1984) J. Exp. Med. **159**, 981
3. Tan, E.M. (1989) Adv. Immunol. **44**, 93
4. Yeo, J.P. & Toh, B.H. (1994) Autoimmunity **18**, 291
5. Mahler, M. et al. (2012) Autoimmunity Rev. **11**, 771
6. Mahler, M. et al. (2010) Lupus **19**, 1527
7. Tzang, B.-S.et al. (1999) Ann. Rheum. Dis. **58**, 630
8. Hsu, T.C. et al. (2006) Clin. Exp. Immunol. **144**, 110
9. Takahashi, T. et al. (1997) Leuk. Lymphoma **28**, 113
10. Vermeersch, P. et al. (2009) Ann. Rheum. Dis. **68**, 1791
11. Fritzler, M.J. et al. (1983) Arthritis Rheum. **26**, 140
12. Moldovan, G.-L. et al. (2007) Cell **129**, 665
13. Brand, S.R. et al. (1994) J. Immunol. **153**, 3070
14. Krishna, T.S. et al. (1994) J. Mol. Biol. **241**, 265
15. Maga, G. & Huebscher, U. (2003) J. Cell Sci. **116**, 3051
16. Huff, J.P. et al. (1990) J. Exp. Med. **172**, 419
17. Tasi, W.-M. et al. (1992) J. Immunol. **149**, 2227

.

AROTEC DIAGNOSTICS