

Datasheet recombinant autoantigens:

PCNA	(Cat. No. 15400) 05/03
P0	(Cat. No. 14100) 05/03
P1	(Cat. No. 14200) 05/03
P2	(Cat. No. 14300) 05/03



DIARECT R&D: New specific tools for SLE diagnosis.

Systemic lupus erythematosus (SLE) is a debilitating, chronic, life-threatening autoimmune disease which can affect virtually any organ or physiologic system in humans. Despite its possible severity SLE is mostly unknown among the average population, because the early stage of this illness can mimic symptoms of minor diseases or disorders, and the diagnosis of SLE is complex. These two factors often hinder early and reliable detection and an exact assessment of SLE prevalence. Estimates of case numbers in the US are as high as 2 million, where in general women are nine times more often affected than men. However, the early and reliable recognition of SLE is crucial for successful therapy.

The course of SLE varies from mild episodic illness to a fatally severe disease. The symptoms emerge in intermittent and unpredictable periods where they are mostly ambiguous and therefore likely to be considered as general discomfort or different diseases: e.g. fatigue, fever, muscle weakness, memory loss, photosensitivity, inflammation, arthritis and pain. SLE is a tissue disease that can affect virtually any organ. Skin, joints, CNS, kidney, lungs, heart, blood and blood-vessels are most frequently affected, but the situation becomes very critical when vital organs are affected, which is likely to happen. Most SLE patients develop an inflammation of the heart (30%), the lungs (40-50%) and half of the patients suffer from lupus nephritis, the major cause of mortality.

The time from SLE-onset to diagnosis could take years, but early recognition is essential to alleviate the progression of SLE. In 1954 only 50% of SLE patients survived a four-year period, while today, with a fairly good management of the disease, 95% of the patients survive a ten-year period. There is currently no cure for SLE, but progression can be controlled, even though therapy is rather unspecific. Treatment normally consists of non-steroid anti-inflammatory drugs, or most often corticosteroids. Severe cases of SLE require the use of cytotoxic substances. All of these drugs have severe side effects, especially in long term use. These therapies clearly improve the progression of SLE but their side effects – in case diagnosis happened late and the dosage has to be high – are the second major cause of death.

SLE like other autoimmune diseases has a multifactorial etiology. In SLE endogenous factors like genetic predisposition or hormonal status, and exogenous factors like drugs, UV-light or viral infection may be implicated. One theory of SLE development starts from an initial apoptosis of lymphoid cells. Their nuclei and nuclear constituents are absorbed by antigen presenting cells which finally results in the expression of antinuclear antibodies (ANA) and antibodies against dsDNA. Antigens and antibodies form immune complexes which cause inflammation and cell damage. During the course of SLE the level of immune complexes increases first in blood, leading to their successive deposition in tissue where they cause organ inflammation and damage. This mechanism causes the diverse facets in the clinical presentation and severity of SLE. Additionally SLE is often accompanied by other autoimmune diseases (overlap syndromes) and therefore requires elaborate diagnostic techniques.

Diagnosis of SLE is a challenge. It comprises medical history, physical examination, biopsy, standard and nonstandard laboratory tests. Serological testing for ANA and anti-dsDNA are of high importance in SLE diagnosis, but even serological diagnosis is a mosaic. Due to the different idiopathic forms of SLE, no nuclear protein is likely to act always as an antigen. Thus new nuclear antigens would further improve diagnosis of SLE, even in its different manifestations. This would also significantly support the development of new therapies.

Many of the common SLE-specific antigens are already in DIARECT's repertoire of recombinant proteins, known for their high purity and lot-to-lot consistency. **Now DIARECT introduces two new classes of recombinant antigens** to extend our set of diagnostic markers: **PCNA** (proliferating cell nuclear antigen) and **ribosomal phosphoproteins P0, P1, P2**, also called P-protein antigens.

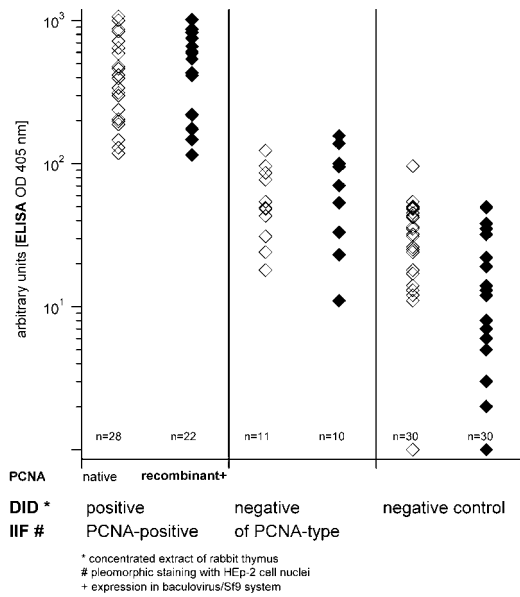
PCNA is a nuclear protein that mediates DNA processing, the regulation of cell cycle and even apoptosis. As a histone-like protein it interacts with many other nuclear proteins and is therefore ubiquitous in caryoplasm. The functional form of PCNA is a ring-shaped trimer, which encircles dsDNA. This explains why PCNA is associated with DNA in absence of a DNA binding motif. PCNA is a highly conserved protein; it even occurs in some viruses. Consequently, if antibodies to PCNA occur in SLE, this antigen is of high diagnostic value because we can expect very small inter-individual variations. PCNA is also known to undergo many conformational changes according to the many interactions with DNA-processing proteins. Thus we can expect the highest diagnostic sensitivity with the native, human form as offered by DIARECT. Preliminary studies confirm the high specificity of DIARECT's recombinant PCNA. Baculovirus-expressed recombinant PCNA is unique within the market and only available from DIARECT.

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Comparison of native and recombinant PCNA in ELISA
 [groups of sera prescreened by double immunodiffusion(DID) and indirect immunofluorescence (IIF) on native PCNA substrates]

(data kindly provided by Prof. R.L. Humbel and P. Schmit, CHL, Luxembourg)



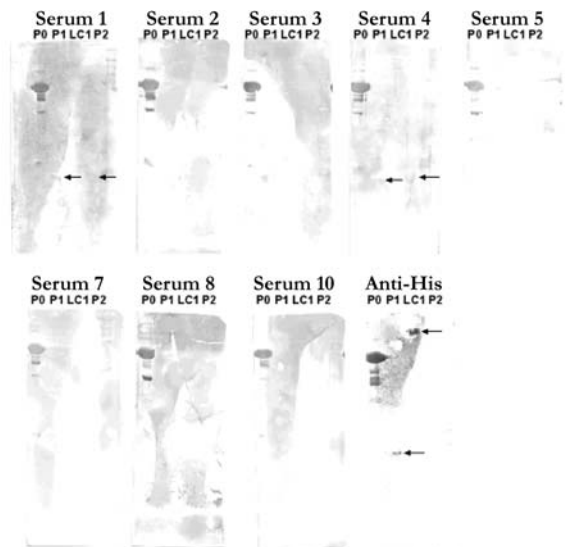
P0, P1, P2 are ribonucleoproteins that are associated with the large ribosomal subunit and therefore common cytoplasmic proteins. Our new P-protein antigens are expressed as full-length proteins. The protein as a whole accomplishes the presentation of the epitope in optimal conformation for antibody detection. This yields a higher prevalence, as compared to the use of a synthetic 20-amino-acid peptide (known to be an important, but not sufficient sequence). Corresponding autoantibodies are present in 10% of SLE sera. If these autoantibodies are to occur in absence of others, it may account for so-called ANA-negative lupus and may thereby fill a gap in SLE diagnosis. Patients with antibodies to ribosomal phosphoproteins have a high frequency of CNS involvement, thus they may be taken as specific markers for an affected brain.

According to our western-blot data based on the ten most specific SLE-sera as determined by an SLE study of 200 SLE patients in Germany we advise:

P0 as key antigen, due to its high specificity,
P1 as a complementary antigen for special cases of SLE,
P2 for scientific purpose.

Western Blots demonstrating the selectivity and specificity of P0, P1, P2

(data from DIARECT R&D)



Western Blots were probed with SLE-positive sera or anti-histidine monoclonal antibody followed by incubation with appropriate secondary antibody-AP conjugates and development with NBT and BCIP. The blots were intentionally overdeveloped to demonstrate the comparatively low reactivity of the sera with P1 and P2 (arrows), whereas P0 is readily detected by all sera tested.

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