



MPO / PR3 Dot (Order No. 4031)

MPO/PR3 Dot is used for the qualitative determination of autoantibodies to the neutrophil cytoplasmic antigens myeloperoxidase (MPO) and proteinase 3 (PR3) in human serum or plasma for the differential diagnosis of systemic vasculitis (SV).

In serological diagnosis of SV anti-neutrophil cytoplasmic antibodies (ANCA) play an important role. These antibodies are usually determined by indirect immunofluorescence (IIF) of ethanol-fixed human neutrophils. Depending on the IIF pattern cytoplasmic ANCA (cANCA) and perinuclear ANCA (pANCA) are distinguished.

MPO is the main antigen in pANCA pattern. MPO autoantibodies occur in a variety of vasculitides such as microscopic polyangiitis, Churg-Strauss syndrome and Polyarteritis nodosa. PR3 has been identified as the main autoantigen causing the cANCA pattern, PR3 autoantibodies are described for Wegener's granulomatosis.

Benefits:

- ★ **No need of sophisticated instruments**
- ★ **80 min incubation**
- ★ **High sensitivity by serum specific cut-off**

Evaluation of results:

The **positive dot control** must score positive in all cases. The coloration of the dot ensures that the test has been run correctly and the kit components are not degraded. If the positive control dot shows no coloration the results should not be interpreted.

The **cut-off control** demonstrates the extent of non-specific antibody binding of the sample in the test. The coloration of the dot corresponds to the minimal intensity above which a sample is considered positive. It might be uncolored even if the test has been run in optimal conditions. The color intensity of the **test dot** depends on the titer of specific antibody binding in the sample. The patient sample is positive concerning a certain antibody if the test dot coloration is stronger (more intense) than the cut-off control

Routine application:

- screening tool for labs with low number of samples
- confirmation of results obtained by IFA or ELISA through an alternative method

KRISHGEN BioSystems



MPO / PR3 Dot (Order No. 4031)

Precision

The Immunodot is a qualitative test and the precision of the assay is evaluated in terms of variation of the visual colour intensity of the dots. The colour intensity is estimated (from 0 to +5) by visual comparison with a reference colour scale (membrane strip with 6 precolored reference dots).

Intraassay variance (n=5)

For each specificity, 3 samples (1 high positive, 1 low positive and 1 negative) were tested in the same run in 5 replicates

Sample	N replicates	Colour intensity of specific Dot
MPO high positive	5	+ 5
MPO low positive	5	+ 1
MPO negative	5	0
PR3 high positive	5	+ 4
PR3 low positive	5	+ 1
PR3 negative	5	0

Interassay variance (n=5)

For each specificity, 3 samples (1 high positive, 1 low positive and 1 negative) were tested on same lot in 5 runs in 1 replicate

Sample	N runs	Colour intensity of specific Dot
MPO high positive	5	+ 5
MPO low positive	5	+ 1
MPO negative	5	0
PR3 high positive	5	+ 4
PR3 low positive	5	+ 1
PR3 negative	5	0

Assay Sensitivity and Specificity

Comparison to reference methods

The evaluation of the kit was performed in collaboration with the *Laboratoire d'Immunopathologie du Centre Hospitalier du Luxembourg (Prof. Dr. R.L. Humbel)*.

Positive MPO (n = 15) and PR3 (n = 40) samples were selected according to Elisa results and specific immunofluorescence patterns on ethanol-fixed human neutrophils. Negative (n = 40) samples are taken from asymptomatic patients and were confirmed negative for MPO and PR3 antibodies by Elisa and immunofluorescence on ethanol-fixed human neutrophils.

Anti-MPO antibodies

	Elisa +	Elisa -	IFA +	IFA -
Dot +	14	1	15	-
Dot -	-	40	-	40
Sensitivity	100 %		100 %	
Specificity	98 %		100 %	

Anti-PR3 antibodies

	Elisa +	Elisa -	IFA +	IFA -
Dot +	33	-	33	-
Dot -	3	44	7	40
Sensitivity	92 %		83 %	
Specificity	100 %		100 %	

KRISHGEN BioSystems