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Dot Blots Analysis in the Separation of Anti-HIV Antibodies.

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1 Works for me dx.doi.org/10.17504/protocols.io.bjm4kk8w

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ABSTRACT

Dot blot frequently is underutilized or is not frequently used because it cannot assess the molecular weight of macromolecules, but it is simple to perform and is useful when a particular biomolecule needs to be identified. In this protocol dot blot analysis is used for detection of anti-HIV antibody [1], and it is also available for determination or separation of any protein. It can be very useful in the immunodiagnosis of infectious diseases caused by viruses [1] and bacteria [2] and in addition for the determination of specific DNA sequences by using probes [3].

References

1. Justiz Vaillant AA, McFarlane-Anderson N, Akpaka PE, Smikle MP, Ramirez N, et al. (2013) Use of Dot Blots Analysis in the Separation of Anti-HIV Antibodies in Animals. J Chromat Separation Techniq 4: 181. doi:10.4172/2157- 7064.1000181

2. Cardona-Castro N, Gotuzzo E, Rodriguez M, Guerra H. Clinical application of a dot blot test for diagnosis of enteric fever due to Salmonella enterica serovar typhi in patients with typhoid fever from Colombia and Peru.Clin Diagn Lab Immunol. 2000;7(2):312-313. doi:10.1128/cdli.7.2.312-313.2000

3. Clément G, Benhattar J. A methylation sensitive dot blot assay (MS-DBA) for the quantitative analysis of DNA methylation in clinical samples.*J Clin Pathol.* 2005;58(2):155-158. doi:10.1136/jcp.2004.021147

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MATERIALS

NAME	CATALOG #	VENDOR
3,3',5,5'-Tetramethylbenzidine	54827-17-7	Sigma Aldrich
Dulbecco's Phosphate Buffered Saline with 2% Fetal Bovine Serum 500 mL	7905	Stemcell Technologies
Nitrocellulose Membrane, Precut, 0.45 µm, 7 x 8.5 cm	1620145	BioRad Sciences
BioDot SF apparatus (Bio-Rad Laboratories Richmond CA USA).		
Peroxidase-labeled HIV proteins; Murex Diagnostics Norcross USA)		

1 Two (2) µl of 1:2 dilutions of chicken IgY or 2 µl of serum from rats and cats is dotted onto nitrocellulose paper. This animal specimens tested positive for the presence of anti-HIV antibodies by ELISA, after being immunized with HIV proteins.

2 Place the nitrocellulose membrane in a BioDot SF apparatus (Bio-Rad Laboratories, Richmond, CA, USA).

3 The membrane is blocked with 5 µL/well of fetal bovine serum with 1%Tris buffer saline.

4 Then 5 µL of a commercial conjugate (peroxidase-labeled HIV proteins; Murex Diagnostics, Norcross, USA) is added and allow to drain by gravity.

5 Finally, 5 µL of the substrate 3,3',5,5'-tetramethylbenzidine (Sigma Chemical Co., St Louis, MO, USA) is added and the mixture is incubated for 20 min.

6 The reaction is stopped by washing the wells with distilled water under a vacuum.

7 The membrane is left to dry.

8 Visualization of color development.

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