

This Week's Citation Classic[®]

Hawkes R, Niday E & Gordon J. A dot-immunobinding assay for monoclonal and other antibodies. *Anal Biochem.* 119:142-7, 1982. [Friedrich Miescher-Institut. Basel. Switzerland]

A procedure for the assay of antibodies in sera based on the application of the antigen as a spot to nitrocellulose filters is described. The method has the merit of being simpler in operation and more sensitive than comparable existing procedures. [The SCI[®] indicates that this paper has been cited in more than 1,765 publications.]

The Dot Immunobinding Assay

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The dot immunobinding assay came about because Andrew Matus, Evelyn Niday, and I needed a rapid and reliable screening assay for the new monoclonal antibody libraries we were generating against the rat cerebellum,¹ and I was proving seriously inept at running the gels for Western blotting.

The plan was simple: we would immunize mice with various rat cerebellar extracts, make hybridomas (then something of a black art), and screen for cell-type specific markers. We were plating our initial fusion into 500 culture wells, so we needed a quick screening assay that could be easily duplicated. At that time, our laboratory was just down the hall from Julian Gordon's group, where Harry Towbin had just invented Western blotting.² It was obvious from the first that this would make a great method to screen hybridoma supernatants, and so I settled down to generate cerebellar

polypeptide blots in quantity. Unfortunately, my gels leaked a lot, which proved frustrating, messy, and irritated everyone around me. It also meant that making, say, 1,000 test blots per experiment was a real undertaking. One day I realized that all we really needed to know was if the particular hybridoma well contained antibodies to anything in the cerebellar extract—exactly which antigens were recognized could come later. This being the case, why not cut out all the electrophoresis and simply apply the sample directly to the nitrocellulose as a small dot? The dot could then be probed with the putative antibody in the hybridoma medium and any binding detected indirectly with an anti-mouse immunoglobulin conjugated to horseradish peroxidase. Luckily, the method worked, and within a couple of weeks we had optimized a screening assay for our hybridomas.

The method seemed so trivial that we never bothered to publish it. Gradually, however, we saw that visitors to the lab were interested, so we drafted a short manuscript. While this was circulating we learned that Julian was developing similar procedures for commercial purposes, so we combined our data to produce the final version. I subsequently modified the technique to assay lectin-binding proteins.³

There are two reasons why the article has been cited so often: On the one hand, the method is easy, low-tech, quantitative, and reliable; on the other hand, I am not the only person who can't run a gel.

The method was reviewed in more detail in 1986.⁴

1. Hawkes R, Niday E & Matus A. Monoclonal antibodies identify novel neural antigens.

Proc. Nat. Acad. Sci. USA 79:2410-1, 1982.

2. Towbin H, Staehelin T & Gordon J. A procedure for the electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets and some applications. *Proc. Nat. Acad. Sci. USA* 76:4350-4, 1979. (Cited 25,025 times.) [See also: Towbin H. Citation Classic[®]. *Current Contents[®]/Clinical Medicine* 16(11): 18, 14 March 1988, and *Current Contents/Life Sciences* 31(11):19, 14 March 1988.]

3. Hawkes R. Identification of concanavalin A-binding proteins after SDS-gel electrophoresis and protein blotting. *Anal. Biochem.* 123:143-6, 1982. (Cited 260 times.)

4.-----The dot immunobinding assay. *Meth. Enzymology* 121:484-91, 1986.

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