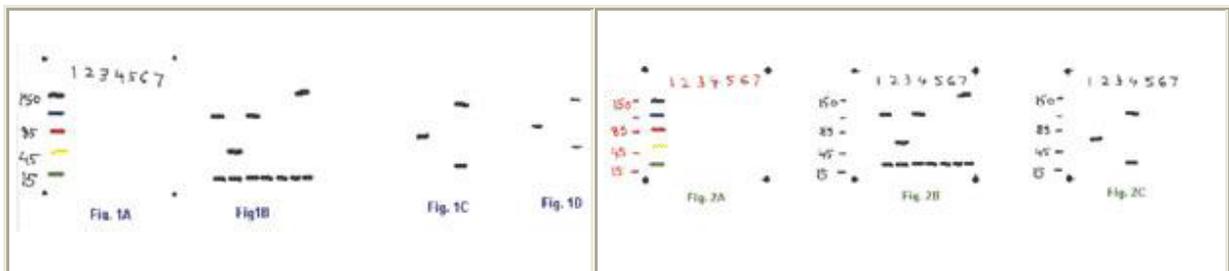




Antigen-Antibody Pens

See what has been missing in Western/Northern/Southern Blots

Western blotting is a common technique to study the purity of samples, size of a given protein, and its approximate concentration in a given protein sample. Typically, proteins are resolved in SDS-gels based upon size and then transferred to blotting membranes (nitrocellulose or PVDF) to probe with specific primary antibodies. A sample lane of known mol. wt. standard protein markers (single color or multi-colors) is also run and transferred along with the samples not only to verify that the transfer of proteins but also to assist in assessing the molecular size of the unknown proteins (Fig. 1A). The positions of various sample lanes can then be marked with a regular pen or pencil (Fig. 1A, markings are 15-150 kDa & lanes 1-7). The membranes are then blocked and probed with primary antibodies, followed by secondary-Ab-enzyme conjugates, and then the colorimetric substrates (e.g., TMB for HRP-conjugates) that produce a color band. The location of the band, as compared to the standard, helps determine the size of the target protein, and the intensity of the band represents its concentration (Fig. 1C). Colorimetric substrates are less sensitive, band/color fades, and it is difficult to document the results.



Problems in conventional Western using ECL-In recent years, highly sensitive enzyme chemiluminescent substrate (ECL) have been developed that emit light, instead of producing color, that can be recorded on x-ray films or more convenient film-less digital imager. However, it has become extremely problematic to keep track of the sample location and the size of the protein bands. As can be seen in Fig.1A, the marking of the protein size (15-150 kda) or the lanes (1-7) by ordinary pen or pencil is lost when results are recorded on X-ray films or digital imager (Fig. 1B). The identity/location of the sample producing the band becomes more problematic when only a few lanes produce the band (Fig. 1C) or when there is a change in the size of the image (Fig. 1D) shows the image smaller; often the image is larger than the original blot) even if one tries to superimpose the image over the original.

Advantages of using the Antigen-Antibody Pens-Using a proprietary and patented technology, ADI has designed and developed specialized fountain pens, called Antigen-Antibody pens-TM, that will allow researchers to deliver or write/mark the blotting membranes in any form or shape or size. A specially formulated antigen-bonded colored dyes helps to see antigen being written on the membrane. One can mark the size of the markers (15-150 kda shown in red-antigen ink, Fig. 2B) or the lanes (1-7 shown in red-ink, Fig. 2B). The blots are processed as usual with the appropriate 2-ab antibody conjugates, and ECL: results recorded on films or digital imager. Unlike the situation in Fig.1C, the use of antigen pens allows the markings to be visible at the end (Fig. 2B or 2C). Therefore, it is possible to see right away, what samples produced results and what the sizes are even if the whole blot image size were to change. There is no need to compare with the original. In addition, Antigen-Antibody Pens are formulated to react only with 2-ab-enzyme conjugates from a give animal species. Therefore, if one is using rabbit primary antibody and the antigen pens specific for rabbit, then the secondary antibody must be anti-rabbit for the Antigen-antibody pens writing to be visible. Any error in the use of wrong conjugate or substrate will produce no writing (control failed) and, of course, the entire blot will be negated (no protein bands in the samples). Therefore, usage of antigen pens provides a built-in positive control for the entire Western procedure. Antigen-Antibody pens are available for various antibody host species.

In addition, the **Antigen Pens are also formulated with Color-coded dyes**, example **Red** dye for **Rabbit**, **Green** dye for **Goat**, and **Black** dye for **Mouse** primary antibodies. Therefore, there will be an added visual control regarding the usage of various Antigen pens. The body of the pen is matched with the antigen dye as well. For example, **Red** pen for **Rabbit**, **Green** Pen for **Goat**, and **Black** pen for **Mouse**.

Salient Features of Antigen-Antibody Pens

- Unique idea and design to allow marking/annotation of blotting membranes with the antigen using natural handwriting-Write on any membrane (Nitrocellulose, PVDF, etc) in any shape, size, number or language.
- Require no other antibodies or reagents-Marked blots are treated the same alongwith the samples.
- Independent of the tag on the 2-ab-The antigen pen for rabbit primary antibodies can be used with any 2-ab-conjugates (HRP, -AP, Biotin labeled). The marking of blots with the Antigen pens is not affected by the blocking agent (milk, BSA, etc) or the presence/absence of any buffer (Tris, PBS or tween).
- Small, convenient, and portable. Store at +4C in unused for several days. Stable at Room Temp.
- Color coded (both the Pen's color and the antigen-dye) for the 2-ab from a given animal specific eliminates mistakes.
- Stable for 6-12 month-Sufficient to mark 100-1000s of blots.
- Provides a built in +ve control-Antigen writing must always give a signal when reacted with an appropriate 2-ab. The absence of signal in the marking/writing of the antigen will indicate the usage of wrong conjugates, insufficient concn or the improper substrate.
- Marking or writing with the antigen pens can be stripped just like the antigens on the blot. The marking will continue to provide the benefit of sample identification even after stripping of blots.

Recommended Usage-Antigen pens are supplied in ready to use form. No preparation is necessary. IN Western blot, Mark/Annotate blotting membranes after the transfer of protein and before blocking. The antigen-dye dries within seconds and blots are ready to process as usual. No special treatment or reagents are required. If marking/writing on fresh membranes, e.g. for dot blot, the pens must be used prior to blocking.

Antigen-Antibody Pens for most Western application

Items Description	Cat #
Antigen-Antibody Pen For Rabbit Primary antibodies	PEN-R1
Antigen-Antibody Pen For Mouse Primary antibodies	PEN-M2
Antigen-Antibody Pen For Goat Primary antibodies	PEN-G3
Antigen-Antibody Pen For Sheep Primary antibodies	PEN-S4
Antigen-Antibody Pen For Chicken Primary antibodies	PEN-C5
Antigen-Antibody Pen For G. Pig Primary antibodies	PEN-P6
Antigen-Antibody Pen For Human Primary antibodies	PEN-H7
Antigen-Antibody Pen For Hamster Primary antibodies	PEN-T8
Antigen-Antibody Pen For Biotin-tagged Primary antibodies (applicable for all species)	PEN-B9

Antigen-Antibody Pens for most Northern and Southern application are also available. The pens have been formulated for procedures that are compatible with most non-radioactive labeling (Biotin, FITC, DNP, tyramide tag) and subsequent detection of DNA/RNA. Please inquire for details and availability.



Antigen-Antibody Pens

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Annotate or Mark Your Blots with an Antigen Pen™

Using the Antigen-Antibody Pens-Using a **proprietary and patented technology**, ADI has designed and developed specialized fountain pens, called Antigen-Antibody Pens™, that will allow researchers to deliver or write/mark the blotting membranes in any form or shape or size.

Features of Antigen-Antibody Pens

- Unique idea and design to allow marking/annotation of blotting membranes with the antigen using natural handwriting-Write on any membrane (Nitrocellulose, PVDF, etc) in any shape, size, number or language.
- Require no other antibodies or reagents-Marked blots are treated the same along with the samples.
- Independent of the tag on the 2-ab-The antigen pen for rabbit primary antibodies can be used with any 2-ab-conjugates (HRP, -AP, Biotin labeled). The marking of blots with the Antigen pens is not affected by the blocking agent (milk, BSA, etc) or the presence/absence of any buffer (Tris, PBS or tween).
- Small, convenient, and portable. Store at 4oC in unused for several days. Stable for routine use at room temp.
- Colour coded (both the Pen's colour and the antigen-dye) for the 2-ab from a given animal specific eliminates mistakes.
- Stable for 6-12 month-Sufficient to mark 100-1000s of blots.
- Provides a built in +ve control-Antigen writing must always give a signal when reacted with an appropriate 2-ab. The absence of signal in the marking/writing of the antigen will indicate the usage of wrong conjugates, insufficient concn or the improper substrate.
- Marking or writing with the antigen pens can be stripped just like the antigens on the blot. The marking will continue to provide the benefit of sample identification even after stripping of the blots.

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