5. intensity of the signal may be higher or lower when using other exposure time or reagents from other vendors. The exposure time should be adjusted accordingly. It is possible to adjust the signal intensity by writing over the same letter or mark or writing with less dye. The signal intensity will also vary with the 2-ab-enzyme conjugate.

6. Immunoblots are exposed to films or digital imaging from just a few seconds to several hours. The exposure time varies greatly with the sensitivity of the ECL or other enzyme substrates. Therefore, any given concentration of the antigen in the pens will provide an adequate exposure time that will be acceptable for various samples. If the signal from the antigen pen marking is not consistent with the samples, it is recommended to take an exposure or the image for the antigen marking (~60-120 seconds) and then a shorter or longer exposure for sample signal.

7. The antigen pens are **color coded** for both the appearance (**pen color**) and the **antigen dye**. For example, Red pen and Red dye are designated for the Rabbit antibodies. Please consult the following chart for the pen's color and antigen dyes.

8. The antigen dye may or may not be able to withstand the washing employed in Western. However, the antigen will remain bound to the membrane irrespective of the visibility of the antigen-dyes. The dyes are neutral and do not by themselves produce any antigen binding or reaction with the antibodies or a signal.

9. Always keep the Pen caps tightly closed when not is use. It is better to store the antigen pens at 4oC, tightly closed in the plastic case unused for days to weeks. The pens are stable at room temp for several hours-days at a time. It is also recommended to bring the pens to room temp before using.

Antigen-Antibody Pens for most Western application-

Antigen-Antibody Pens For the following **species Primary antibodies** **	**Cat #**	**Pen Color**	****To use with the 2-Ab**
Rabbit	PEN-R1	Red	Anti-Rabbit IgG (HRP/AP etc)
Mouse	PEN-M2	Black	Anti-Mouse IgG (HRP/AP etc)
Goat	PEN-G3	Green	Anti-Goat IgG (HRP/AP etc)
Sheep	PEN-S4	**White**	Anti-Sheep IgG (HRP/AP etc)
Chicken	PEN-C5	Pink	Anti-Chicken IgG (HRP/AP)
G. Pig	PEN-P6	Blue	Anti-G. Pig IgG (HRP/AP etc)
Human	PEN-H7	**Orange/ blue**	Anti-Human IgG (HRP/AP)
Hamster	PEN-T8	Yellow/P urple	Anti-Hamster IgG (HRP/AP)
Biotin tagged (applicable for all species)	PEN-B9	Blue/whi te	Streptavidin-Conjugates (HRP/AP/FITC etc)

-Example, If **primary antibodies are made in **Rabbit**, you must use **Rabbit pen** and **anti-rabbit IgG-conjugates** (HRp or AP or Biotin or FITC etc) as the secondary antibody.

Antigen- Pens are available for Northern/Southern applications that are compatible with most non-radioactive labeling (Biotin, FITC, DNP, etc tag) and subsequent detection of DNA/RNA.

Antigen-Antibody Pens-™ **-

See what has been missing in Western Blots

Instruction Manual for

Antigen-Antibody Pen For **Rabbit** Primary antibodies	**Cat #** PEN-R1
Antigen-Antibody Pen For **Mouse** Primary antibodies	**Cat #** PEN-M2
Antigen-Antibody Pen For **Goat** Primary antibodies	**Cat #** PEN-G3
Antigen-Antibody Pen For **Sheep** Primary antibodies	**Cat #** PEN-S4
Antigen-Antibody Pen For **Chicken** Primary antibodies	**Cat #** PEN-C5
Antigen-Antibody Pen For **G. Pig** Primary antibodies	**Cat #** PEN-P6
Antigen-Antibody Pen For **Human** Primary antibodies	**Cat #** PEN-H7
Antigen-Antibody Pen For **Hamster** Primary antibodies	**Cat #** PEN-T8
Antigen-Antibody Pen For **Biotin**-tagged Primary antibodies	**Cat #** PEN-B9

**-Patent Pending

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LIFE TECH Life Technologies™
Your Molecular & Cell Technology Partner

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.

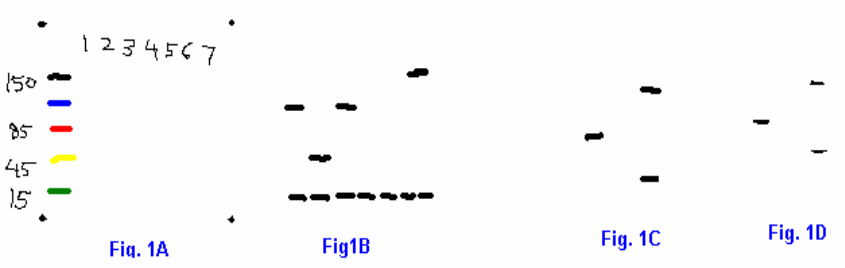

Fig. 1A Fig1B Fig. 1C Fig. 1D

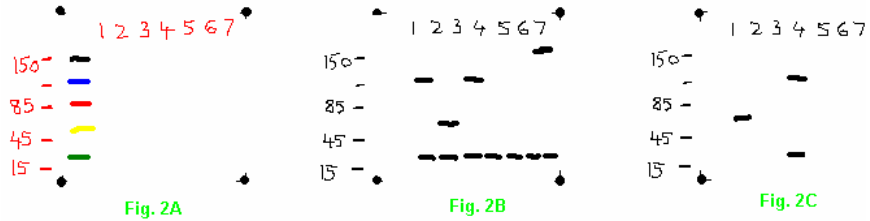

Fig. 2A Fig. 2B Fig. 2C

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. The antigen in the pens is designed to react with a given 2-ab or the 2-ab-enzyme conjugates. 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, usage of antigen pens provides a built-in positive control for the entire Western procedure.

Antigen-Antibody pens are available for various antibody and host species. The Antigen Pens are also formulated with Color-coded, antigen-bonded 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.

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.
- **Requires 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 if unused for several days. Stable for routine use 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 the blots.

Recommended Usage-

1. Antigen pens are supplied in ready to use form. No preparation is necessary. Make sure the antigen-ink is not dried up. A gentle flick should get the ink flowing again.

2. In Western blot, **write/Annotate blotting membranes after the transfer of protein and before blocking.** Remove excess of water by drying with a clean paper towel or blotting paper and then write with the antigen pens. The excess liquid on the membrane may smudge or smear the writing. The antigen-dye dries within seconds and blots are ready to block as usual. No special treatment is required.

3. Writing over the membrane that have already been **pre-blocked with BSA/Milk** or any other agent may not produce good writing and deposition of the antigen.

4. The antigen in the pens is formulated to produce a **good signal in approx 60 seconds** when using colorimetric or ECL-Western blotting kit from ADI. The