

Directions for use of *Antibody Amplifier*TM:

Updated 8.1.2008

Key notes:

1. Each *Antibody Amplifier*TM has 12 wells (1 slide per well), and therefore holds 12 slides.
2. Each well in the *Antibody Amplifier*TM requires approximately 3 mls of solution. This volume may alarm some scientists, but using the *Antibody Amplifier*TM on a standard laboratory rocker [will improve overall antibody efficiency up to 13,333x](http://www.prohisto.com) (see www.prohisto.com for examples), so the scientist actually uses LESS antibody per slide. Money is SAVED.

Example calculation:

	Standard procedure	<i>Antibody Amplifier</i> TM **
Antibody dilution =	1:100	1:10,000
Total liquid to cover tissue =	200 μ l	3000 μ l
Amount antibody per tissue =	2 μl	0.3 μl

**Each antibody may have a different change of efficiency with the *Antibody Amplifier*TM. Based on the above calculations, the change in efficiency can be as low as 15x. In this case, for both the standard procedure and the use of the *Antibody Amplifier*TM, the scientist will need 2 μ l of antibody. Most antibodies we have tested so far have provided an improved efficiency of over 15x, and therefore for most antibodies, you will use LESS with the *Antibody Amplifier*TM.

3. The *Antibody Amplifier*TM is designed to be stackable, and therefore, an experiment can be carried out with multiple *Antibody Amplifiers*TM. We suggest, however, that you secure them together with laboratory tape.
4. Following placement of slides into the *Antibody Amplifier*TM, the chamber with the lid on is placed on a standard laboratory rocker during incubation.
5. The *Antibody Amplifier*TM is chemical resistant (e.g. to xylenes).
6. The scientist can decide either to use the *Antibody Amplifier*TM during the entire procedure, or alternatively only during specific parts of the assay. **In all cases, however, the scientist should use the *Antibody Amplifier*TM during antibody incubation.**
7. Slides can be taken out of chambers with any pipette tip or forceps.
8. For more information, or trouble-shooting, please see our website (www.prohisto.com), or call 803-407-0506.

Use of the *Antibody Amplifier*TM during a standard immunohistochemistry procedure using paraffin-embedded tissues:

As described in the above section, the scientist can decide either to use the *Antibody Amplifier*TM during the entire procedure, or alternatively only during specific parts of the assay. **In all cases, however, the scientist should use the *Antibody Amplifier*TM during antibody incubation.**

1. Deparaffinize slides (optional to use the *Antibody Amplifier*TM).
 - If the scientist decides to use the *Antibody Amplifier*TM during this step, it is safe to do so, as the *Antibody Amplifier*TM is xylene and ethanol resistant.
2. Wash (optional to use the *Antibody Amplifier*TM)
3. Antigen retrieval
4. Block (optional to use the *Antibody Amplifier*TM)
5. Primary antibody (**use the *Antibody Amplifier*TM**)
 - Each well requires approximately 3 mls to cover the slide. This is significantly more than the standard procedure, but as described in our brochure, the primary antibody can be diluted significantly more than in standard protocols. This depends on the antibody, and tissue. In most cases, the scientist will end up using LESS antibody per tissue section. Please see our website (www.prohisto.com) for examples.
6. Place the *Antibody Amplifier*TM on a standard rocker, place the lid on the chamber, and incubate while rocking.
 - *If you choose to re-use the antibody, it can be stored at -20°C, and in most cases can be re-used up to 4 times without loss of antibody efficiency.
7. Wash (optional to use the *Antibody Amplifier*TM).
8. Secondary antibody (**use the *Antibody Amplifier*TM**)
 - Note: The secondary antibody is usually tagged to allow for an amplification step.
 - Note: You can dilute the secondary antibody significantly more with the ***Antibody Amplifier*TM**... the amount of dilution is dependent on the source of the secondary, but in our experience, secondaries can be diluted out 20 to 100 fold or more compared to the sedentary method..
9. Wash (optional to use the *Antibody Amplifier*TM).

10. Amplifying step (e.g. avidin-biotin complex/HRP conjugate) (optional to use the *Antibody AmplifierTM*).

Note: You can dilute the amplifier significantly more with the *Antibody AmplifierTM* ... the amount of dilution is dependent on the source and type of amplification.

9. Wash (optional to use the *Antibody AmplifierTM*).

10. Substrate

11. Counter-stain

Please go to www.prohisto.com for general IHC troubleshooting links.