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DATA SHEET

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> PDS Over Time

PDS OVER-Time allows multiple extended exposures thanks to a longer-lasting output signal. PDS OVER-Time allow detection of HRP using photographic or other imaging methods. Blots can also be repeatedly exposed to film to obtain optimal results or stripped of the immunodetection reagents and reprobed. The sensitivity, intensity and duration of the signal allow for detection of HRP using photographic or other imaging methods. Blots can also be repeatedly exposed to film to obtain optimal results or stripped of the immunodetection reagents and reprobed.

cat. no.	*amount*	*note*
STS-E500	250ml	enhancer solution
	100ul	hydrogen peroxide 30%

FOR RESEARCH USE ONLY

SHIPPING
Shipped at room temperature.

STORAGE
Store at 4°C

SHELF LIFE
12 months

FORM
liquid

RECOMMENDED PROCEDURE
hydrogen peroxide and enhancer solution at a 3ul :10ml ratio.

Optimization and notes:

- For best results, it is essential to OPTIMIZE all components of the system including sample amount, primary and secondary antibody concentration, and the choice of membrane and blocking reagents.
- Because no blocking reagent is optimal for all systems, empirical testing is essential to determine the appropriate blocking buffer for each Western blot system. Determining the proper blocking buffer can help increase sensitivity and prevent nonspecific signal caused by cross-reactivity between the antibody and the blocking reagent. Furthermore, when switching from one substrate to another, a diminished signal or increased background sometimes results because the blocking buffer was not optimal for the new system.
- Use sufficient volumes of wash buffer, blocking buffer, antibody solution and substrate working solution to cover blot and ensure that it never becomes dry. Using large blocking and wash buffer volumes minimizes nonspecific signal.
- For optimal results, use a shaking platform during incubation steps
- Add Tween®-20 (final concentration of 0.1-0.2%) to the blocking buffer and all diluted antibody solutions to minimize nonspecific signal.
- Do not use sodium azide as a preservative for buffers. Sodium azide is an inhibitor of HRP and could interfere with this system.
- Do not handle membrane with bare hands. Always wear gloves or use clean forceps
- Exposure to the sun or any other intense light can harm the substrate. For best results avoid prolonged exposure to any intense light. Short-term exposure to typical laboratory lighting will not harm the working solution.

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PROCEDURE SUMMARY

Primary Antibody Diluition Range: 1:1000 - 1:5000
Secondary Antibody Diluition Range: 1:10000 - 1:100000

- Prepare the working solution by mixing the two substrate components (peroxide solution and enhancer solution) at a 3ul:10ml ratio.

Note: Exposure to the sun or any other intense light can harm the substrate. For best results avoid prolonged exposure to any intense light. Short-term exposure to typical laboratory lighting will not harm the working solution.

- Incubate blot 1 min. in the working solution.
- Drain excess reagent. Cover blot with clear plastic wrap.
- Expose blot to X-ray film.

Detailed Western Blotting Procedure:

- Remove blot from the apparatus and block nonspecific sites with blocking buffer* for 60 minutes at room temperature (RT) with shaking.
- Rinse with wash buffer* and add the appropriate primary antibody diluited in blocking buffer. Incubate for 1-2 hours with shaking. If desired, blots may be incubated with primary antibody overnight at 2-8°C.
- Rinse the membrane in wash buffer with shaking for ≥ 10 minutes. Replace wash buffer at least 3 times. Increasing the wash buffer volume and/or the number of washes may help reduce background.
- Incubate blot with the appropriate HRP-conjugate secondary antibody diluited in blocking buffer for 1-2 hour at RT with shaking.
- Repeat whashes to remove non-bound HRP-conjugate.
- Prepare working solution by mixing equal parts of peroxide and enhancer solution. Use 0.1 ml working solution per cm2 of membrane.
- Incubate blot in the working solution.
- Drain excess reagent. Cover blot with clear plastic wrap. Use an absorbent tissue to remove excess liquid and to carefully press out any bubbles from between the blot and surface of the membrane protector.
- Place the protected membrane in a film cassette with the protein side facing up. Turn off all lights except those appropriate for film exposure (e.g., a red safelight).
-Carefully place a piece of film on top of the membrane. A recommended first exposure time is 30 seconds. Exposure time may be varied to achieve optimal results. Enhanced or pre-flashed film is not necessary.

Note: The exposure time may be varied to achieve optimal results. If the signal is too intense, reduce exposure time or optimize the system by decreasing the antigen and/or antibody concentrations.

- Light emission is most intense during the first 5-30 minutes after substrate incubation. Light emission will decrease with time.
- Blots may be stripped and reprobed if necessary.

*Supported buffers:

blocking buffer A : 1xTBS, 0,1% Tween®-20, 3% low fat dry milk
blocking buffer B : 1xPBS, 0,2% Tween®-20, 5% low fat dry milk
washing buffer A : 1xTBS, 0,1% Tween®-20
washing buffer B : 1xPBS, 0,2% Tween®-20