

## HRP-Juice/ HRP-Juice PLUS (1 03 14/ 1 03 15)

### Components included:

<b>HRP-A</b>	<b>50ml</b> <b>Store at +4-8°C</b>
<b>HRP-B</b>	<b>50ml</b> <b>Store at +4-8°C.</b>

Applications: Western Blot, ELISA

Mix equal volumes of HRP-A and HRP-B in a clean container and equilibrate at room temperature for 30 minutes before use. Best results for chemiluminescence can be obtained from 2 minutes to 20 minutes after contacting HRP-Juice with HRP enzyme.

### Standard Protocol for Blotting with Secondary Antibody-HRP Conjugates

Separate proteins by electrophoresis.

Transfer proteins to the membrane.

Block the membrane with blocking buffer.

Incubate the membrane with primary antibody for 30 to 45 minutes.

Wash the membrane with washing buffer.

Incubate the membrane with HRP conjugated secondary antibody for 30 to 45 minutes.

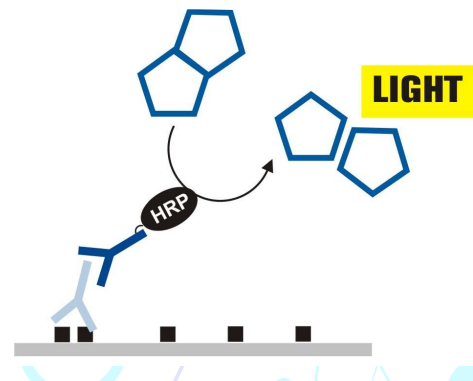
Wash the membrane with washing buffer 5 to 6 times.

Incubate the membrane with HRP-Juice for 2 to 3 times.

Drain the excess substrate from the membrane.

Wrap the membrane in a plastic.

Expose the membrane to film or take a picture with CCD camera.



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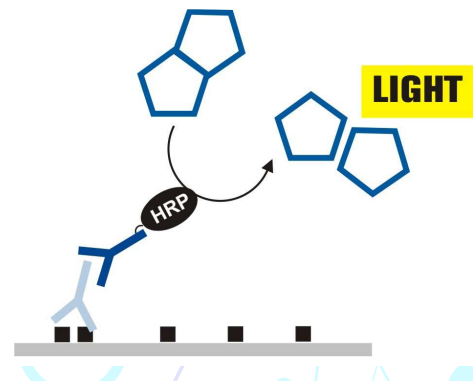
Wash the membrane with washing buffer 5 to 6 times.

Incubate the membrane with HRP-Juice for 2 to 3 times.

Drain the excess substrate from the membrane.

Wrap the membrane in a plastic.

Expose the membrane to film or take a picture with CCD camera.



## HRP-Juice/ HRP-Juice PLUS (1 03 14/ 1 03 15)

### Standard Protocol for Blotting with Streptavidin-HRP Conjugates

Separate proteins by electrophoresis.

Transfer proteins to the membrane.

Block the membrane with blocking buffer.

Incubate the membrane with primary antibody for 30 to 45 minutes.

Wash the membrane with washing buffer.

Incubate the membrane with biotin labeled secondary antibody for 30 to 45 min.

Wash the membrane with washing buffer.

Incubate the membrane with Streptavidin-HRP conjugate for 30 to 45 minutes.

Incubate the membrane with HRP-Juice for 2 to 3 times.

Drain the excess substrate from the membrane.

Wrap the membrane in a plastic.

Expose the membrane to film or take a picture with CCD camera.

### **Example for detection of T4 by ELISA PROCEDURE:**

Place the required number of anti-T4-Ab coated wells in to the well holder.

Pipette the 50  $\mu$ l of T4 standards (0, 2, 5, 10, 15 & 25  $\mu$ g/dl), samples and controls into the designated wells.  
Pipette the 75  $\mu$ l of working enzyme conjugate reagent and place into each well.

Shake the plate on vibrator or on appropriate mixer for 20-30 seconds.

incubate the plate in the oven at room temperature for 30 minutes.

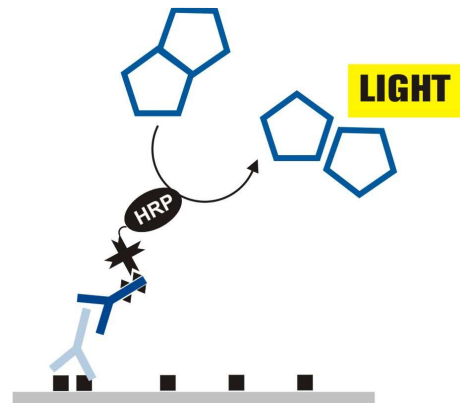
Remove the mixture from the wells by flicking the plate into a waste container.

Wash the wells four times with dilute Wash buffer.

Dry the wells containing plate on paper towel by striking.

Now add the 125  $\mu$ l HRP-Juice into each well and shake the plate on the vibrator for at least 10 seconds.

Keep the plate inside the sample chamber of luminometer and start taking the reading after five-minute interval.



## HRP-Juice/ HRP-Juice PLUS (1 03 14/ 1 03 15)

### Standard Protocol for Blotting with Streptavidin-HRP Conjugates

Separate proteins by electrophoresis.

Transfer proteins to the membrane.

Block the membrane with blocking buffer.

Incubate the membrane with primary antibody for 30 to 45 minutes.

Wash the membrane with washing buffer.

Incubate the membrane with biotin labeled secondary antibody for 30 to 45 min.

Wash the membrane with washing buffer.

Incubate the membrane with Streptavidin-HRP conjugate for 30 to 45 minutes.

Incubate the membrane with HRP-Juice for 2 to 3 times.

Drain the excess substrate from the membrane.

Wrap the membrane in a plastic.

Expose the membrane to film or take a picture with CCD camera.

### **Example for detection of T4 by ELISA PROCEDURE:**

Place the required number of anti-T4-Ab coated wells in to the well holder.

Pipette the 50 µl of T4 standards (0, 2, 5, 10, 15 & 25 µg/dl), samples and controls into the designated wells.  
Pipette the 75 µl of working enzyme conjugate reagent and place into each well.

Shake the plate on vibrator or on appropriate mixer for 20-30 seconds.

incubate the plate in the oven at room temperature for 30 minutes.

Remove the mixture from the wells by flicking the plate into a waste container.

Wash the wells four times with dilute Wash buffer.

Dry the wells containing plate on paper towel by striking.

Now add the 125 µl HRP-Juice into each well and shake the plate on the vibrator for at least 10 seconds.

Keep the plate inside the sample chamber of luminometer and start taking the reading after five-minute interval.

