# T-Pro LumiDura Chemiluminescent Substrate Kit ( for HRP )

Store at 2~8°C

(JT96-K006S) 100 ml *2
(reagent A 125ml + reagent B 125ml)
(JT96-K006M) 250 ml *2
(reagent A 250ml + reagent B 250ml)

T-Pro Biotechnology

**This product is for laboratory research ONLY and not for diagnostic use.**

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| **Description** | The principle of T-Pro LumiDura Chemiluminescent Substrate Kit is based on chemiluminescent and is very convenient to detect the Horseradish peroxidase (HRP) activity in many assays such as Western blotting，Southern and Northern. HRP catalyze the chemiluminescent oxidation of cyclic diacylhydrazides such as luminol by hydrogen peroxide ($H_2O_2$). T-Pro LumiDura Chemiluminescent Substrate Kit can enhance the luminol-dependent chemiluminescent and can be wildly use to detect the present of HRP-conjugated antibodies or streptavidin which binding to antigen or nucleotide sequence respectively. |
| **Comparison** | Signal Duration: 12 hours<br>Detection Method: X-ray film or imaging acquisition system<br>Suggested Antibody Dilution: Primary: 1/1,000 – 1/10,000<br>Secondary: 1/50,000 – 1/250,000<br>Lower Detection Limit: Low-Picogram ($10^{-11}$)<br>High- Femtogram ($10^{-14}$) |
| **Storage** | T-Pro LumiDura Chemiluminescent Substrate Kit is stable for 2~8°C |

## Procedural

1. Mix the reagent A 1：1 with reagent B in T-Pro LumiDura Chemiluminescent Substrate Kit and incubate the mixture for 1 min in room temperature.
2. Add the sufficient mixture solution to cover the membrane (0.1ml/$cm^2$). Incubate the membrane for 1 min in room temperature.
3. Discard the excess mixture in membrane and wrap the membrane in saran wrap. Carefully and gently remove the air bubbles from the membrane.
4. Place the membrane in the film cassette and keep the protein side up. Turn off the lights and use safety light. Then place a sheet of film on the membrane and close the cassette and expose for 10-90 seconds.
5. Open cassette and transfer the exposed film to developing machine. Then place a new film on the membrane and expose again.
6. The exposure time of second film can be adjusted by the intensity of first film.
7. If the intensity was too high, please wait up to 10 minutes before re-exposing.

## TROUBLE SHOOTING

| Problem | Possible Cause and Solution |
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| **A.High Background** | |
| High Concentration for antibody | *Optimize/Apply the lower concentration of antibody. |
| The gathered secondary antibody | *Apply 0.2um nylon membrane / change fresh secondary antibody |
| The incubated temperature are too high when process the antibody | *Incubated at 4°C |
| Secondary antibody has happened nonspecifically bind or cross reaction with Blocking Solution | *Set the control group for Secondary antibody (not added primary antibody)<br>*To go down the concentration of secondary antibody |
| Primary antibody or Secondary antibody cross reaction with Blocking Solution | *Add the Tween-20 into the wash buffer when processed the incubation, to avoid the cross reaction. |
| Unsuitable Blocking Solution | *Choice and apply the difference Blocking Solution |
| Not completed on the blocked | *Choice/Optimize Blocking Solution<br>*On Blocking Solution to Increase the concentration of protein<br>*Optimize the time and temperature when incubation<br>(Incubate 2 hrs. keep at RT ,if you would like to incubate for overnight, please keep at 4°C)<br>*To add Tween-20 to Blocking Solution and final concentration at 0.05%<br>*To add Tween-20 to diluted antibody and final concentration at 0.05% |
| Not enough time on blocked process | *To extend the blocked time.<br>*Choice and change suitable Blocking Solution. (skim milk, BSA & serum) |
| Antibody cross reaction with the other proteins | *Choice and apply the difference Blocking Solution and do not apply nonfat dried milk to block on the membrane in the system of Biotin/avidin.<br>* To go down the concentration of secondary antibody<br>*To test and inspection the cross reaction between the membrane and secondary antibody. |
| Not completed on the wash | *To extend the wash time and increase used volume of wash buffer<br>*To add Tween-20 to wash buffer and keep the concentration at 0.05% |
| Too long for Exposed time | *To short the exposed time |
| The trouble on membrane | *Use clean tweezer and glove when process<br>*Change a new membrane<br>*Apply enough solution and keep to wet on membrane anytime<br>*To avoid the overlapping membrane and cover each other<br>*Becareful and do not damage the membrane |
| Not completed on the wash of membrane | *To wash the membrane frequency |
| To apply unsuitable membrane | *NC membrane has lower background compared with PVDF |
| The membrane is too dry | *Make sure the enough solution, to avoid the dry membrane |
| The pollution of buffer | *To fill the flesh buffer<br>*To filer the buffer |
| The pollution of instrument | *Make sure to keep clean for all instruments<br>*Make sure no remaining gel on membrane |
| **B.Lower signal/No signal** | |
| Not completed on the process for transferring of membrane | *The efficiency of transfer membrane has determined on gel after the process of transferring.<br>*Make sure it was completed activity between gel and membrane when the process of transferring.<br>*Apply gel and membrane on one filter paper, and do not recycle. It should be has a correct and complete assemble on electrophoresis process<br>*To process the membrane following the protocol<br>*To avoid the high temperature when electrophoresis<br>*Apply the positive control group or pre- dye Marker<br>*Ideal transferring time and electric current<br>*Make sure the sample do not damage when process |
| Not completed on assembling of Protein and membrane | *Add 20% methanol to buffer of transfer membrane.<br>*Apply a small-bore / low molecular weight membrane |
| Antibody | *Increase the concentration of antibody, the efficiency of assemble was bad for antibody and antigen, antigen lost the activity. |
| Not enough antigen | *Increase the volume of 1xsample |
| Antigen cover by Blocking Solution | *Try to apply difference Blocking Solution.<br>*Ideal the proteinic concentration in Blocking Solution.<br>*To short the blocking time. |
| The Blocking Solution with | *Remove NaN3. |

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| NaN3 | |
| The short Exposed time | *To extend the exposed time |
| The short incubation time for substrate | *At least 5 mins. |
| The gelation for protein on membrane | *Some of Blocking Solution maybe result in the active degradation on protein. |
| The biodegradation has happen during the process of stored protein | *Re-prepare new sample |
| The concentration was too low for Primary antibody or and Secondary antibody | *Increase the concentration of antibody, and extend the incubate time. |
| Primary antibody or Secondary antibody cross reaction with Blocking Solution | *Use the Tween-20 when blocking or change the Blocking Solution (skim milk, BSA, serum and gel in common usage). |
| The sample without target protein or the lower target protein on sample (unefficient antibody) | *Set the positive control group. If it run a absolute result for control group, and the sample maybe has not including target protein or the contents of target protein too low.<br>For the lower target protein, please increase the 1xsample to 20-30 ug per well at least, and apply Protease inhibitor when prepare sample., or extract target protein by classification. |
| Not completed on the process for transferring of membrane, or overuse on the wash of membrane | *To test the efficiency of transfer membrane by Ponceau S, the PVDF membrane need to soak completed and following the correct process when transferring, do not overuse on the wash of membrane. |
| Over-blocking | *To use 0.5% skim milk or thinner for antibody, or change the Blocking Solution and reduce the time of blocking. |
| Inefficiency for primary antibody | *Apply flesh antibody, and split up into a couple of small package for storage.<br>*No repeated freezing and thawing. |
| The secondary antibody control by NaN3 | *To avoid all solution and container to contain the NaN3(The inhibitor for HRP) |
| Inefficiency for enzyme or substrate | *Mix the enzyme and substrate directly. The enzyme has no reaction if it could not coloration.<br>*Use flesh and active enzyme and flesh substrate. |
| Membrane do not soak completed | *Use 100% methanol to soak through membrane. |
| Molecular weight for target protein are less than 10,000 | * Apply a small-bore / low molecular weight membrane<br>*To short the transferring time. |
| The concentration of methanol are too high | *The high concentration of methanol will resulting the divided for protein and SDS and then precipitate on gel, in the meanwhile the gel will become hard and traction. The high molecular weight protein will be inhibited in transferring.<br>*Please decrease the concentration of methanol or apply alcohol or isopropanol to instead. |

## C.Nonspecific band

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| SDS nonspecific combine to protein on membrane | *Wash completed after transferring<br>*Do not use SDS |
| The protein of sample has degraded | *Use flesh prepare sample and apply Protease inhibitor |
| Antibody do not for purification | *Use single clone or antibody with purification |
| The concentration was too high for Primary antibody | *Decrease the concentration of primary antibody under the situation for keeping sensitive. |
| High volume for 1xsample | *Reduce and adjust the volume for 1xsample. |