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Product Information

79454 Ampliflu Red Western Blot Kit

Content

- 90101 Ampliflu Red (5 vials)
- 41640 DMSO (3 ml)
- 79382 PBS (5 tablets)
- 49674 H_2O_2 solution (6 ml)
- Technical information sheet

Product Details

Spectral data:	λex = 571 nm / λem = 585 nm
Content:	Sufficient for 50 applications
Sensitivity:	LOD: 1 ng/band, but may vary depending on the antibodies used

Application

Classical immunoblotting for protein detection on a membrane is often based on horseradish peroxidase (HRP) labeled secondary antibodies. Peroxidase can be detected using colorimetric substrates, or chemiluminescence-based substrates with CCD camera or X-ray film detection.
The new Ampliflu Red Western blot Kit contains the peroxidase substrate Ampliflu Red, that is enzymatically converted to the highly fluorescent compound, resorufin. Fluorescent imaging is performed on the immunoblot membrane using a laser scanner with an excitation wavelength of 571 nm and an emission filter of 585 nm. Other imaging systems with similar excitation sources and emission filter settings may also be used.
When working with Ampliflu Red, standard protocols for the immunoblotting-procedure can be used until the detection step. The membrane is incubated with a mixture of Ampliflu Red and H_2O_2 in PBS for 5 minutes. The imaging can then be done via laser-excitation and an emission filter. No extra time for signal accumulation is necessary as it is for chemiluminescent signals. The use of Ampliflu Red is suitable for protein quantities between 1 ng and 1 µg per band of protein.
In order to receive an optimal signal to background ratio, it is strongly recommended to use a low-fluorescence PVDF-membrane for the Western blot transfer and a 5 % BSA blocking solution.

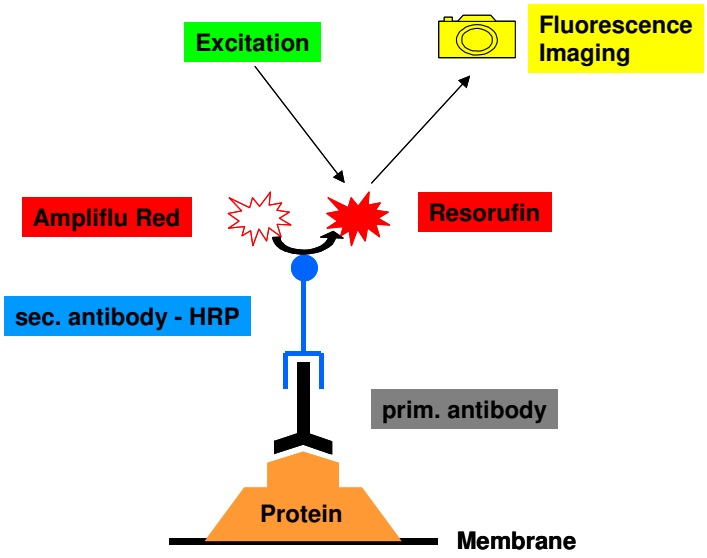

Figure 1. Schematic overview for the use of Ampliflu Red. A protein is immobilized by Western blot transfer after SDS-PAGE. The protein is bound with a primary antibody and followed by a secondary antibody carrying a horseradish peroxidase (HRP) label. The membrane is incubated in a solution containing Ampliflu Red and hydrogen peroxide. Ampliflu Red is converted into resorufin by the action of HRP. The resultant fluorescent signal can be detected with excitation at 571 nm and emission at 585 nm.

Protocol

For membranes in the mini-format, the blocking and washing steps are performed in a volume of 50 ml, whereas, the antibody incubations are done in 25 ml.

1. Perform Western blot transfer onto a low-fluorescence PVDF-membrane (e.g. 05317)
2. Prepare PBS solution: Dissolve 1 PBS-tablet in 200 ml H_2O
3. Block with 5 % BSA in PBS (1 h – overnight)
4. Short rinse with PBST (PBST contains 0.1 % TWEEN® 20 in PBS solution)
5. Incubate with primary antibody (2 – 3 h)
6. Wash with PBST (3 x 5 min)
7. Incubate with HRP-labeled secondary antibody (1 h). Note: The activity of HRP is inhibited by azide!
8. Wash with PBST (3 x 5 min)
9. Short rinse with PBST
10. Prepare Ampliflu Red solution: Dissolve the content of 1 vial Ampliflu Red in 200 µl DMSO
11. Create staining solution (total volume 2 ml):
    - 1880 µl PBS
    - 20 µl Ampliflu Red solution
    - 100 µl H2O2 solution
12. Incubate WB-membrane in staining solution (5 min)
13. Fluorescent imaging

Detection

Peroxidase converts Ampliflu Red into resorufin, which has an excitation maximum at λex = 571 nm and an emission maximum at λem = 585 nm. Fluorescence detection can be performed by illumination on a laser-scanner (e.g. Fuji® FLA-3000, with 532 nm excitation and 580 nm emission filter). Other imaging systems are possible with similar excitation sources and emission filter settings. Try to minimize the exposure to light.

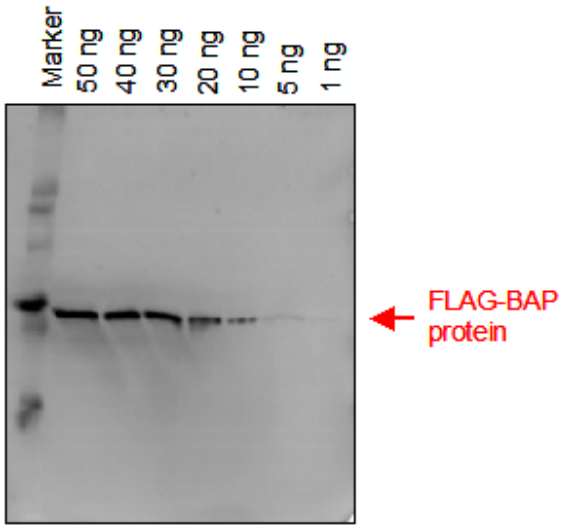

Figure 2. Detection of FLAG-BAP™ protein (50 ng – 1 ng) by immunoblotting using the Ampliflu Red Western blot Kit on Immobilon™-FL PVDF-Membrane. The membrane was blocked using 5 % BSA in PBS solution and then incubated with monoclonal ANTI-FLAG® M2 antibody developed in mouse (Cat. No. F1804, diluted 1:1000). The secondary antibody used was anti-mouse-IgG-peroxidase developed in rabbit (Cat. No. A9044, diluted 1:10,000). Imaging was performed on a FLA-3000 Fuji® laser scanner with an excitation wavelength of 532 nm and with a 580 nm emission filter.

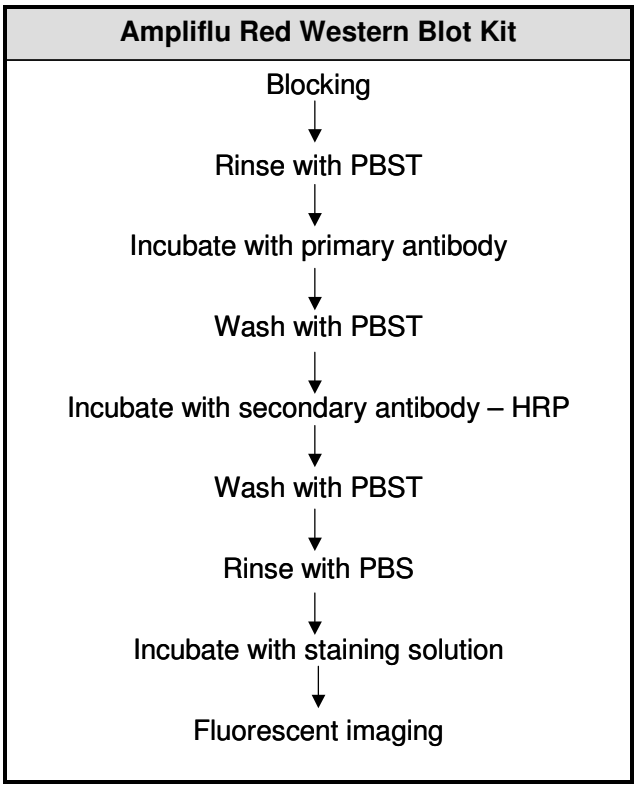

Figure 3. Schematic protocol for the Ampliflu Red Western Blot Kit.

Storage / Handling
Please store kit at room temperature. After dissolution in DMSO, Ampliflu Red should be stored at 4 °C in the dark. After that, it should be used up within 1 month. Do not expose to light unnecessarily. Reuse of the staining solution is not recommended.

Related Products

Cat. No.	Description	Package size
90101	Ampliflu Red	5 mg
05317	Immobilon™-FL PVDF membrane	10 ea
P5927	Tween® 20	500 ml
05480	BSA	10 / 100 / 500 g

Precautions and Disclaimer:
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.