For life science research only.
Not for use in diagnostic procedures.

rGFP
from jellyfish *Aequorea victoria*, recombinant expressed in *E. coli*

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Recombinant Green Fluorescent Protein

Cat. No. 11 814 524 001	50 µg 50 µl, 1 mg/ml

Store the product at +2 to +8°C.

1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Content
1	rGFP, Recombinant Green Fluorescent Protein	▪ Purified from *E. coli.* ▪ 1 mg/ml in buffer solution containing 5 mM Tris-HCl, 5 mM EDTA (pH 8.0).	1 vial, 50 µg

1.2. Storage and Stability

Storage Conditions (Product)

When stored at +2 to +8°C, the product is stable through the expiry date printed on the label.

Vial / Bottle	Label	Storage
1	rGFP	Store at +2 to +8°C.

1.3. Additional Equipment and Reagent required

For western blotting

See section, **Working Solution** *for additional information on preparing solutions.*

- Standard electrophoresis equipment
- Transfer buffer
- Methanol
- Western Blocking Reagent*
- PVDF Western Blotting Membranes*
- Anti-GFP*
- PBS*
- Tween 20*
- Nonfat dry milk
- Anti-mouse IgG (H+L)-POD
- Lumi-Light Western Blotting Substrate*
- Plastic wrap
- Lumi-Film* Chemiluminescent Detection Film*

1.4. Application

The purified rGFP produces a single major staining band and can be used as a:

- Positive control in detecting GFP fusion proteins by SDS-PAGE and western blot analysis.
- Standard for experiments involving fluorescence microscopy.

2. How to Use this Product

2.1. Before you Begin

Working Solution

Solution	Preparation
Anti-GFP working solution	Dilute 10 µl of Anti-GFP* concentrate with 10 ml (1:1,000) of a 1:20 dilution of Western Blocking Reagent* in PBS. *This volume provides sufficient antibody for a 10 cm × 10 cm PVDF membrane*.*
Anti-mouse IgG (H+L)-POD working solution	Prepare 10 ml by diluting anti-mouse IgG (H+L)-POD 1:3,000 in PBS containing a final concentration of 2.5% nonfat dry milk.
Detection solution	See Instructions for Use of the Lumi-Light Western Blotting Substrate*.
Transfer buffer	10% methanol, 24 mM Tris base, and 194 mM glycine.

2.2. Protocols

Polyacrylamide gel electrophoresis

rGFP can be used as a standard for SDS-PAGE applications.

① For a standard mini-gel apparatus, load 1 µg total rGFP protein per lane for gels stained with Coomassie blue dye.

② For SDS-PAGE and western blotting applications, rGFP protein can be diluted and solubilized by boiling for up to 5 minutes in standard solubilization buffers.

Western blotting

See section, **Working Solution** *for additional information on preparing solutions.*
The following method has been developed specifically for the Anti-GFP antibody and the GFP control.
For optimal sensitivity of detection, use the Anti-GFP with PVDF membranes*, and the Lumi-Light Western Blotting Substrate*.*

1. For standard mini-gel apparatus, load 5 ng total rGFP protein per lane to obtain a strong positive signal on blots exposed for 1 minute using chemiluminescent detection.
   For detection of GFP or GFP fusion proteins in crude cellular lysates, as much as 50 µg of total lysate protein can be loaded per lane.
2. Perform electrophoresis according to standard protocols.
3. Wet a PVDF membrane in 100% methanol.
   – Equilibrate the membrane in Transfer buffer.
   – Perform western transfer to the PVDF membrane.
4. Block the membrane using gentle rotation for 1 hour at +15 to +25°C in a 1:10 dilution of Western Blocking Reagent* diluted in phosphate-buffered saline (PBS: 1 mM KH_2PO_4, 10 mM Na_2HPO_4, 137 mM NaCl, 2.7 mM KCl; pH 7.0).
   10 ml of working-strength Western Blocking Reagent provides sufficient volume to cover a 10 cm × 10 cm PVDF membrane.*
5. Incubate the blocked membrane with the Anti-GFP working solution for 1 hour at +15 to +25°C under gentle rotation.

6. Rinse the membrane with PBS containing 0.1% Tween 20* (PBST).

7. Wash the membrane 2 × 10 minutes with PBST.

8. Add the anti-mouse IgG (H+L)-POD secondary antibody preparation (10 ml) to the blot.
   – Incubate the blot for 1 hour at +15 to +25°C under gentle rotation.

9. Rinse membrane with PBST.

10. Wash 3 × 10 minutes with PBST.

11. Following the protocol described with the Lumi-Light Western Blotting Substrate*, add that reagent set's Detection solution to the membrane.
    – Incubate the membrane for 1 minute.

12. Drain excess Detection solution from the membrane.
    – Wrap the membrane in plastic wrap.

13. Expose the membrane to X-ray film or Lumi-Film Chemiluminescent Detection Film* in a film cassette for 60 seconds according to the method provided with the Lumi-Light Western Blotting Substrate.
    Substrate development and X-ray film exposure conditions required to achieve optimal signals may vary for each experiment.

3. Additional Information on this Product

3.1. Test Principle

Green fluorescent protein is a spontaneously fluorescent protein originally isolated from the jellyfish *Aequorea victoria*.

- Molecular cloning of the GFP gene and its subsequent expression in heterologous systems have established recombinant GFP as a valuable reporter molecule for *in vivo* visualization of gene expression events in a wide variety of cell types and organisms.
- Since rGFP requires no additional substrates or cofactors, rGFP fluorescence can be easily detected using blue or UV light after expression in either prokaryotic or eukaryotic cells.

Preparation

A 27-kD protein isolated from transformed *E. coli*, rGFP is highly purified, but maintains the intrinsic fluorescence of native GFP. The absorption and fluorescence emission spectra for rGFP are identical to those of the GFP isolated from *Aequorea victoria*.

3.2. Quality Control

For lot-specific certificates of analysis, see section **Contact and Support**.

4. Supplementary Information

4.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

4.2. Changes to previous version

Layout changes.
Editorial changes.

4.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Anti-GFP	200 µg	11 814 460 001
PVDF Western Blotting Membranes	1 roll, 30 cm x 3.00 m	03 010 040 001
Tween 20	50 ml, 5 x 10 ml	11 332 465 001
Buffers in a Box, Premixed PBS Buffer, 10x	4 l	11 666 789 001
Western Blocking Reagent, Solution	100 ml, 10 blots, 100 cm^2	11 921 673 001
	6 x 100 ml, 60 blots, 100 cm^2	11 921 681 001
Lumi-Film Chemiluminescent Detection Film	100 films, 8 x 10 inches, 20.3 x 25.4 cm	11 666 657 001
Lumi-Light Western Blotting Substrate	1 kit, 400 ml (4 x 100 ml), 40 or 400 blots with 10 cm x 10 cm	12 015 200 001

4.4. Trademarks

All product names and trademarks are the property of their respective owners.

4.5. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products.

4.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

4.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

4.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

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