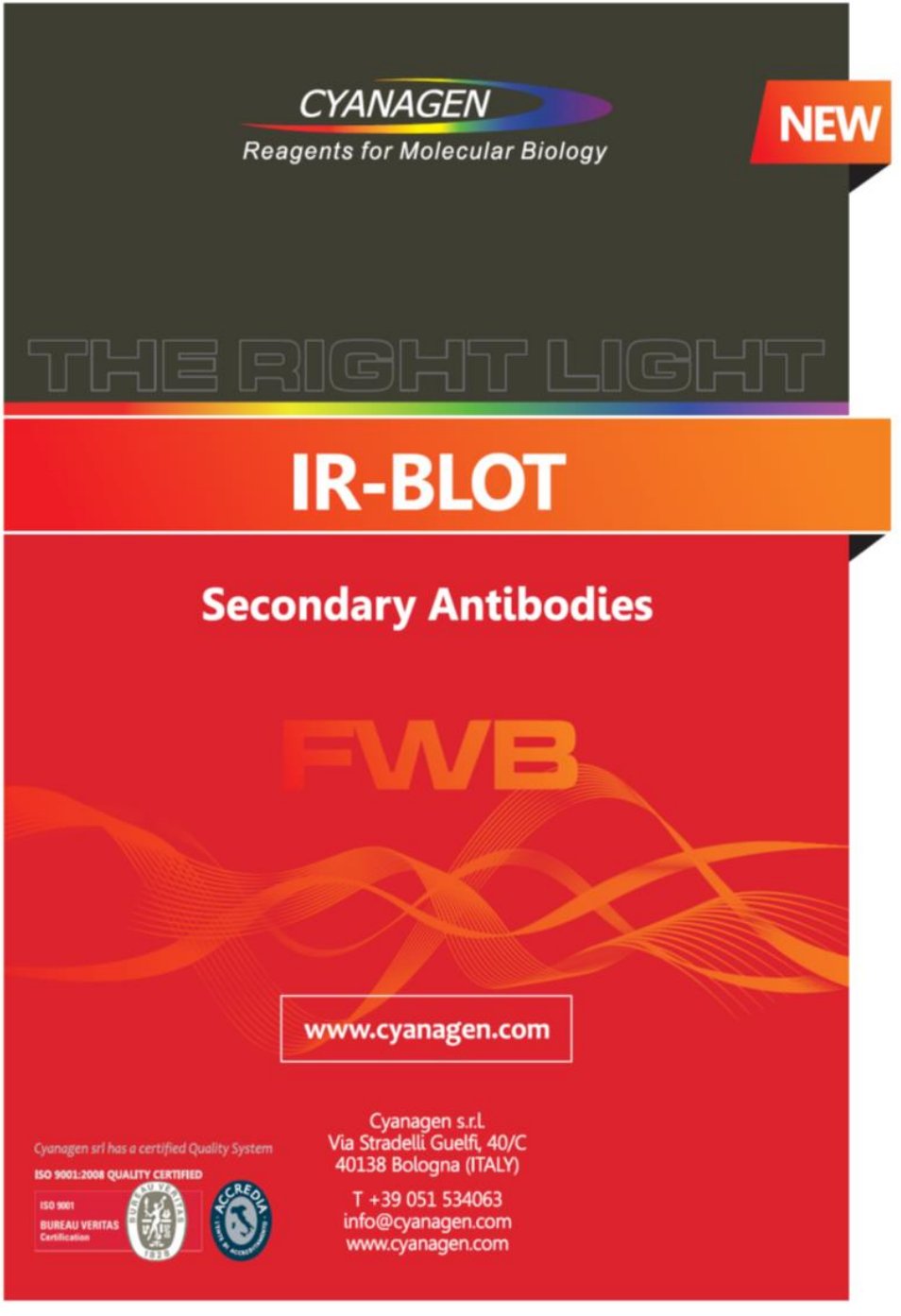

IR-Blot Secondary antibodies Rev01

About us

Cyanagen is a biotech company located in Bologna, dedicated to research, development and production of reagents for molecular diagnostic since 2003 and one of the leading companies in the field of reagents for Western blotting and Elisa.
The main product lines are focused on chemiluminescence and fluorescent dyes for biological analysis, genomics, proteomics and chemical sensors.
They are based on Cyanagen internationally patented technologies and achieve outstanding performance in terms of sensitivity and stability.
The products are extremely versatile and perfectly suited to the latest analytical instrumentation. These products are also available as OEM.

Cyanagen s.r.l. has a certified Quality System

ISO 9001-2008 QUALITY CERTIFIED

Product manual

IR-BLOT

Secondary Antibodies Optimized for Multicolor Infrared Fluorescent Western Blotting

IR-BLOT ARE INTENDED FOR RESEARCH USE ONLY AND SHALL NOT BE USED IN ANY CLINICAL PROCEDURES OR FOR DIAGNOSTIC PURPOSES.

www.cyanagen.com

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1. Introduction

IR-Blot secondary antibodies are high quality secondary antibodies optimized for quantitative western blotting in the infrared region. These antibodies are highly cross-adsorbed, so ensuring extremely low background and very specific signal.
The high fluorescence intensity of IR-Blot secondary antibodies allows a very high sensitivity and an optimal signal to noise ratio, both in the 700 nm and 800 nm channel.
In order to provide the best value for money, IR-Blot secondary antibodies are optimized for multiplex infrared western blotting and for lower optimal working dilution.
IR-Blot secondary antibodies can be used on instruments with excitation and emission filters in the infrared region.

Features:

- Highly cross-adsorbed antibodies
- High sensitivity and low background
- Quantitative Western blotting
- Optimized for multicolor IR-Western blotting

IR-Blot secondary antibodies are available as Goat anti-Mouse and Goat anti-Rabbit conjugates.

2. Properties

Antibody amount: 0.1 mg

Antibody concentration: 1.0 mg/mL after reconstitution

Antibody	Excitation wavelength	Emission wavelength
IR-Blot 700 – Goat anti Mouse (H+L) IgG	678 nm (in PBS)	690 nm (in PBS)
IR-Blot 700 – Goat anti Rabbit (H+L) IgG	678 nm (in PBS)	690 nm (in PBS)
IR-Blot 800 – Goat anti Mouse (H+L) IgG	774 nm (in PBS)	790 nm (in PBS)
IR-Blot 800 – Goat anti Rabbit (H+L) IgG	774 nm (in PBS)	790 nm (in PBS)

Recommended dilution:

Application	Recommended	Suggested Range
Fluorescence Western Blotting	1:25000	1:15000 – 1:50000*

*Optimal dilution will vary and should be determined empirically.

For better results, dilute the IR-Blot 700 secondary antibodies in TBS-T or PBS-T with 0.01% of SDS and wash using TBS-T or PBS-T with 0.005% of SDS.

Form of Antibody: secondary antibody is lyophilized in phosphate buffer saline, pH 7.4. Contains 10 mg/mL BSA as stabilizer and 0.01% sodium azide as preservative, after reconstitution.

Reconstitution and storage:
Protect from light. Store at +4°C prior to reconstitution. Reconstitute the content of the vial with 0.1 mL of ultrapure water. Mix gently by inverting and allow to rehydrate for at least 30 minutes before use. Centrifuge product if particulates are seen in the solution after standing at room temperature. Once reconstituted, the product is stable for up to 3 months at +4°C.

Follow universal safety precautions.

3. Other materials required

- **Running Buffer**
- **Transfer Buffer**
- **Blotting membranes,** PVDF or nitrocellulose
- **Blocking Buffer** (non-fat dried milk or BSA in PBS or TBS)
- **Primary antibodies**
- **Tween® 20**
- **PBS/TBS***
- **Ultrapure water**
- **Methanol for wetting of PVDF**
- **SDS**

*Either PBS-based and TBS-based buffers can be used with this protocol.
If you use a PBS-based buffer system, use PBS for blocking buffer and be sure to use PBS also when preparing antibody dilutions and washing buffer.
If you use a TBS-based buffer system use TBS for blocking, antibody dilutions, and washes.

NOTE: if you are not satisfied with your results, please refer to troubleshooting section.

4. Protocol

Optimal transfer conditions, membrane type, blocking agent washing buffer and antibody dilutions may vary in different applications. It is crucial to optimize all these parameters for best results with high signal and low background.

1. After membrane transfer:
   PVDF:
   a) Wet the membrane with methanol 100% and incubate for 1 minute
   b) Rinse with water
   c) Wet in PBS or TBS for 2 minutes
   Nitrocellulose:
   a) Wet the membrane with PBS or TBS and incubate for 2 minutes

   Do not write on the membrane with a pen. Most pen inks are fluorescent in the 700 nm wavelength.
   Always use clean forceps to handle the membrane.

IMPORTANT! Use membrane with low autofluorescence. Before beginning your Western Blot, be sure your membrane has a low level of autofluorescence by imaging it (wet and dry) in the 700 and 800 channels.

2. Place the membrane with protein side up into a fresh tray with your choice of **Blocking Buffer** (e.g. 3% non-fat dry milk in TBS-T or PBS-T Buffer). Incubate the membrane in Blocking Buffer for 30÷60 minutes with gentle agitation on a rocker/shaker. A maximum blocking time of 2 hours at RT should not be exceeded. Blocking for too long can result in antigen masking and loss of protein.

IMPORTANT! During blocking do not use detergents. To reduce background, dilute your blocking agent in PBS or TBS without Tween®20.

3. Rinse the membrane twice with **TBS-T/PBS-T Buffer**.

4. Dilute the primary antibody in fresh **TBS-T or PBS-T Buffer** to the suggested primary antibody dilution.

5. Incubate the membrane with protein side up in the primary antibody solution for 1 to 2 hours at RT. To increase sensitivity, try an overnight incubation at 4°C with agitation on a rocker. Make sure the membrane is completely covered with **TBS-T or PBS-T Buffer** with primary Ab.
   NOTE: Add 3% non-fat dry milk in TBS-T or PBS-T Buffer when dilute Ab to reduce non specific bindings. Milk contains many proteins which bind to the membrane. Proteins contained in the milk bind to the membrane and fill potential nonspecific sites. After this, when you incubate with your antibody, it binds to the antigen and has less possibilities of nonspecific binding. If you are working with anti-phosphoproteins the adding of milk is not appropriate. Use 3% BSA instead.

6. Wash the membrane with protein side up 3 times quickly and 4 times for 5 minutes each with **TBS-T or PBS-T Buffer** with gentle agitation on a shaker. **After each washing place the membrane on a new clean tray with fresh TBS-T or PBS-T buffer.**

IMPORTANT! Washing buffer should always contain 0.1% Tween®20. Wash the IR-Blot 700 secondary antibodies using TBS-T or PBS-T with 0.005% of SDS.

7. Dilute the IR-BLOT secondary Ab in fresh **TBS-T or PBS-T Buffer** to the suggested secondary antibody dilution. Suggested dilution ranges from 1:15000 to 1:50000. Optimal dilution will vary and should be determined empirically. Incubate the membrane with protein side up for 30 minutes to 1 hour at RT in a tray covered with aluminum foil. Increasing the incubation time of the secondary antibody usually leads to higher background. For 1-color western blot and for maximizing sensitivity, prefer IR-Blot 800 secondary antibodies.

IMPORTANT! To reduce background, add detergents to your diluted antibody. Optimal detergent concentration may vary and should be optimized for each application. Tween®20 can be used at a concentration between 0.1 and 0.2% in diluted antibody when using nitrocellulose and at 0.1% for PVDF. Dilute IR-Blot 700–secondary antibodies in TBS-T or PBS-T with 0.01% of SDS.

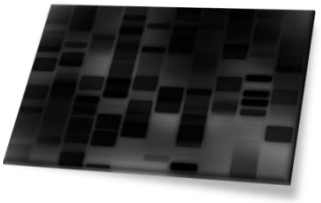
8. Wash the membrane with protein side up 3 times quickly and 4 times for 5 minutes each with TBS-T or PBS-T Buffer with gentle agitation on a shaker. After each washing place the membrane on a new clean tray with fresh TBS-T or PBS-T buffer. For better results, wash the IR-Blot 700 secondary antibodies using TBS-T or PBS-T with 0.005% of SDS.

9. After this, wash 2 times quickly with PBS or TBS. Your membrane is ready to image.

5. Hints for multiplex detection

- Incubate the two primary antibodies together in the same diluted antibody solution. Be sure to use primary antibodies from different host species (e.g. one from rabbit and one from mouse)
- Incubate the two secondary antibodies together in the same diluted antibody solution.
- Choose IR-Blot 800 secondary antibodies to detect your low-abundant protein to have low background and high sensitivity.
- Prefer IR-Blot 700 secondary antibodies for your housekeeping protein or your more abundant protein.
- Always use highly cross-adsorbed secondary antibodies to avoid cross-reactivity.
- Acquire the fluorescence signal using either laser or LED-based imaging device with 700 and 800 channels.

6. Troubleshooting



High membrane background

Membrane autofluorescence. Use membrane with low autofluorescence. Before beginning your Western Blot, be sure your membrane has a low level of autofluorescence by imaging it (wet and dry) in the 700 and 800 channels.

High concentration of Ab. Further dilute primary and secondary Ab. Follow suggested Ab dilutions.

Inefficient blocking. Do not include detergents during the blocking step. To reduce background, dilute your blocking agent in PBS or TBS without Tween®20.

Insufficient washing. Increase both the volume, length and number of wash steps. Always use sufficient volumes to submerse the membrane. For better results, washing buffer should always contain 0.1% Tween®20. Wash the IR-Blot 700 – secondary antibodies using TBS-T or PBS-T with 0.005% of SDS.

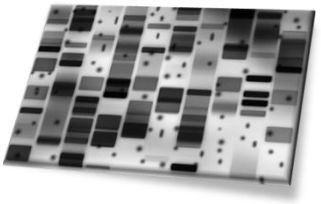
Primary antibody is not specific for the protein of interest. Use monospecific or antigen affinity purified antibodies. Dilute primary antibody in PBS-T or TBS-T. Always incubate your primary antibody at 4°C overnight and not at room temperature. Use monospecific or antigen affinity purified Ab.

Non-specific binding of secondary antibody. Add detergents to your diluted antibody. Optimal detergent concentration may vary and should be optimized for each application. Tween®20 can be used at a concentration between 0.1 and 0.2% in diluted antibody when using nitrocellulose and at 0.1% for PVDF. Dilute IR-Blot 700 – secondary antibodies in TBS-T or PBS-T with 0.01% of SDS.

Poor handling of membrane. Be sure to handle the membrane only with clean forceps and non-powdered gloves.

Contaminated buffer solutions. Check buffers for particulate or bacterial contaminate. Replace old buffers.

Presence of traces of Coomassie Blue. Do not use trays with contamination of Coomassie Blue. Coomassie Blue fluoresces in 700 channel detection. Always use clean tray for incubations.



Irregular fluorescent spots

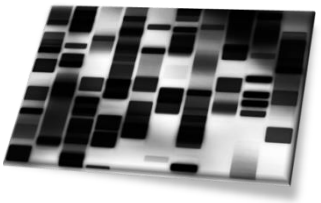
Air bubble trapped in membrane. Remove air bubbles by gently rolling a clean pipette or a test-tube during sandwich assembling.

Unevenly hydrated membrane. Make sure that the membrane is fully immersed during washes and antibody incubations.

Contaminated equipment. Protein or pieces of gel remaining on the unit may stick to the membrane. Antibody can get trapped in the gel, and then are washed out poorly, resulting in intense localized signal.
Aggregation of blocking agent. When blocking agent is powder stir it over night at 4°C to make sure it is completely dissolved.
Interaction of the membrane with sample tray. Always use clean plastic trays to avoid any type of cross-reaction.

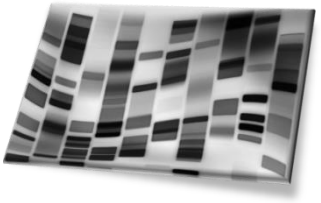
No bands or weak bands

Inefficient transfer. Ensure that there is good contact between membrane and gel during sandwich assembling. High MW protein may require more time for transfer. Reduce voltage or time of transfer for low molecular weight proteins (< 10 kDa).
Antibodies may have lost activity. Perform a Dot Blot. Follow manufacturer's recommended storage and avoid freeze/thaw cycles.
Incorrect secondary antibody used. Confirm host species/Ig type of primary Ab.
Low protein-antibody binding. Reduce the number of washes to minimum.
Non-fat dry milk may mask some antigens. Decrease blocking time. Decrease milk percentage in Blocking Buffer or substitute with 3% BSA Blocking Buffer.



Non-specific bands

Aggregation of analyte. Increase amount of reducing agent to ensure complete reducing of disulfide bonds.
SDS interference. The presence of SDS may result in the development of unspecific bands caused by antibodies binding to the charged SDS molecules associated with the proteins. Wash thoroughly the membrane after transfer with water.
High protein concentration. A commonly seem effect is the diffusion of protein bands. Reduce the amount of protein initially loaded.
Primary antibody is not specific for the protein of interest. Use monospecific or antigen affinity purified antibodies. Dilute primary antibody in PBS-T or TBS-T. Always incubate your primary antibody at 4°C overnight and not at room temperature. Use monospecific or antigen affinity purified Ab.



Uneven or jagged bands

Uneven gel run. Load all available wells. Empty wells can be loaded with sample buffer.

Voltage or current were too high during electrophoresis. Reduce voltage or current during electrophoresis.

Effects of high salt in samples. Reduce NaCl concentration in TBS-T/PBS-T Buffer (100mM÷350mM).

7. Ordering information

PRODUCT	ORDER-NO.	UNIT SIZE
IR-Blot 700 Goat anti-Rabbit	F2H111,S001	0,1mg
	F2H111,S005	5 x 0,1mg
IR-Blot 700 Goat anti-Mouse	F2H112,S001	0,1mg
	F2H112,S005	5 x 0,1mg
IR-Blot 800 Goat anti-Rabbit	F1L107,S001	0,1mg
	F1L107,S005	5 x 0,1mg
IR-Blot 800 Goat anti-Mouse	F1L108,S001	0,1mg
	F1L108,S005	5 x 0,1mg

For further information visit **www.cyanagen.com**

For orders: call **+39 051.534063**
mail to **sales@cyanagen.com**

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