

Applicable to: **2view™Goat anti-Rabbit [Gold/HRP]** 5000-0000, **2view™Goat anti-Mouse [Gold/HRP]** 5001-0000, **2view™Donkey anti-Goat [Gold/HRP]**: 5002-0000.  
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## INTRODUCTION

Expedeon's 2view™ is an innovative and unique staining method optimized for western blot applications, consisting of a ternary complex of 40nm InnovaCoat® Gold nanoparticles, horseradish peroxidase (HRP) and a secondary antibody.

A common problem in western blotting is that the amount of protein of interest in the sample is usually unknown, thus the detection method that is used may turn out to be insufficiently sensitive or unnecessarily complicated. 2view™ affords two levels of sensitivity: if the analyte is present in high amount (nanogram level) a red band, given by the gold label, will appear on the membrane without the need to perform more complex chemiluminescent detection; if the analyte is present in low amount (picogram level) and the red band is not clearly displayed, it is still possible to check whether the analyte is present by detecting the HRP activity with ECL (Figure 1). The components of the ternary complex are covalently bonded, preventing the desorption of proteins from the gold particle and maximizing the signal-to-noise ratio.

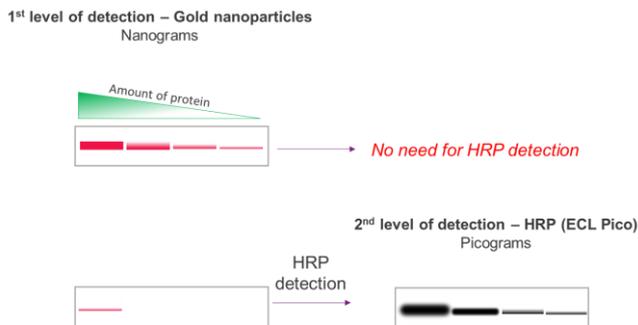


Figure 1.

The sensitivity of the ternary complex is low nanogram level, and it increases up to 1000 times with ECL, allowing the detection of picogram level of analyte while keeping the background low. Expedeon's 2view™ is available for anti-rabbit, anti-mouse and anti-goat primary antibodies.

## KIT CONTENTS

- 10ml 2view™

## SHIPPING CONDITIONS

The kit is shipped at ambient temperature. Please store the 2view™ at -20°C upon receipt.

## INSTRUCTIONS

Before starting, select the 2view™ secondary antibody according to the species of the primary antibody being used.

Although western blot protocols may vary, they all follow some common basic steps. The instructions reported below are guidelines

and some of the steps require optimization depending on the sample and primary antibody being used.

1. Resolve the protein mixture by gel electrophoresis;
2. Blot the separated molecules onto a nitrocellulose or polyvinylidene difluoride (PVDF) membrane;
3. Optional: to check if the transfer was complete stain the gel with *InstantBlue* (ISB1L); alternatively stain the membrane reversibly with Ponceau S;
4. Block the membrane with 1x TBS, 0.05-0.1% Tween20®, 5% BSA (non-fat dry milk, casein, fish skin gelatin may be used instead of BSA) for at least one hour on gel rocker in order to prevent any nonspecific binding. Make sure the membrane is fully covered with the buffer and does not dry during the incubation;
5. Probe the membrane with the primary antibody diluted in blocking buffer at 4°C O/N on a gel rocker. Check the primary antibody dilution range in the supplier datasheet. Make sure the membrane is fully covered with the buffer during the incubation;
6. Rinse the membrane three times with 1x TBS, 0.05-0.1% Tween20® for 5 minutes to remove any unbound primary antibody;
7. Incubate the membrane for one hour on a gel rocker with 2view™ diluted 1:25 in blocking buffer. Longer incubation time does not affect negatively the result, on the contrary binding may improve although the background could increase too and longer wash steps may be required. Make sure the membrane is fully covered with the buffer during the incubation.
8. Rinse the membrane three times with 1x TBS, 0.05-0.1% Tween20® for 5 minutes to remove any unbound 2view™ complex;
9. Check your membrane for any red bands;
10. If red bands are not visible perform the chemiluminescent detection with ECL. HRP detection has been optimized for use with *ECL Pico* (ECLP0500, ECLP0250). For other ECL detection buffer see FAQs.

## STORAGE OF CONJUGATES

After use, 2view™ diluted in blocking buffer can be stored at 4°C for 2 weeks and re-used for a maximum of 3 times.

## RELATED PRODUCTS

Electrophoresis Equipment  
[www.expedeon.com/products/proteomics/gel-electrophoresis/electrophoresis-equipment](http://www.expedeon.com/products/proteomics/gel-electrophoresis/electrophoresis-equipment)

RunBlue Protein Gels

[www.expedeon.com/products/proteomics/gel-electrophoresis/runblue-protein-gels](http://www.expedeon.com/products/proteomics/gel-electrophoresis/runblue-protein-gels)

Buffers & Accessories for gel electrophoresis  
[www.expedeon.com/products/proteomics/gel-electrophoresis/buffers-accessories](http://www.expedeon.com/products/proteomics/gel-electrophoresis/buffers-accessories)

InstantBlue Protein Stain  
[www.expedeon.com/products/proteomics/gel-electrophoresis/instantblue-stain](http://www.expedeon.com/products/proteomics/gel-electrophoresis/instantblue-stain)

RunBlue Tris Glycine SDS Blot Buffer  
[www.expedeon.com/products/proteomics/western-blotting/buffers-membranes/runblue-tris-glycine-sds-blot-buffer](http://www.expedeon.com/products/proteomics/western-blotting/buffers-membranes/runblue-tris-glycine-sds-blot-buffer)

RunBlue Tris-Glycine Blot Buffer  
[www.expedeon.com/products/proteomics/western-blotting/buffers-membranes/runblue-tris-glycine-blot-buffer](http://www.expedeon.com/products/proteomics/western-blotting/buffers-membranes/runblue-tris-glycine-blot-buffer)

RunBlue Blot Membrane  
[www.expedeon.com/products/proteomics/western-blotting/buffers-membranes/runblue-blot-membrane/](http://www.expedeon.com/products/proteomics/western-blotting/buffers-membranes/runblue-blot-membrane/)

## TECHNICAL SUPPORT

For technical enquiries get in touch with our technical support team at: [www.expedeon.com/contact/](http://www.expedeon.com/contact/)