



YamayBio

# **ECL Western Blotting UltimateSensitivity Substrate (Femto)**

QUICK START GUIDE

Research use only.  
Not for diagnostic procedures.

# Contents and storage

ECL Western Blotting Ultimate Sensitivity Substrate (Femto)

VQ4726-100ml

VQ4726-500ml

Storage: Store at 2~8°C.

## Introduction

ECL Western Blotting Ultimate Sensitivity Substrate (Femto) is a chemiluminescent HRP substrate designed to optimize light emission for mid-femtogram sensitivity. Its prolonged luminescence signal is ideal for both X-ray film and digital imaging systems.

## Quick Start Protocol

1. **Post-Immunodetection:** Keep the immunodetected membrane moist with wash buffer.
2. **Substrate Preparation:** Mix the two components of the substrate kit in equal volumes. Prepare 0.1 ml of solution per square centimeter of membrane. For standard membrane sizes, use 7 ml for mini-membranes (7 x 8.5 cm) and 12 ml for midi-membranes (8.5 x 13.5 cm). **No incubation period is necessary.** Detect the membrane directly following thorough mixing.
3. **Signal Detection:** Visualize the chemiluminescent signal using a digital imaging system or X-ray film.

# Optimization Tips

Observation	Cause	Solution
Nonspecific bands	Low protein degradation.	<ul style="list-style-type: none"> <li>Minimize sample freeze-thaw cycles;</li> <li>Extract proteins at low temperatures in the presence of protease inhibitors;</li> </ul>
	Sample overload.	Load less sample.
	Poor antibody specificity.	Opt for a specific antibody.
	Insufficient blocking.	Increase blocking time or use Protein-Free Blocking Buffer(1×) (Cat. No. BM3152).
High background	Concentration of antigen or antibody is too high.	Decrease amount of antigen or antibody.
	Too much HRP in the system.	Further dilute the HRP-conjugate.
	Inadequate blocking.	Optimize blocking conditions.
	Inappropriate blocking reagent.	Try a different blocking reagent.
	Inadequate washing.	Increase length, number or volume of washes.
	Poor milk powder dissolution resulted in particles adhering to the membrane.	Ensure complete milk powder dissolution.
No reaction or weak signal	Too much HRP in the system depleted the substrate and caused the signal to fade quickly.	Remove excess wash buffer from the membrane to ensure peroxidase is at optimal pH.
	Insufficient quantities of antigen or antibody.	Increase antibody concentration or incubation times.
	Low protein abundance or degradation.	<ul style="list-style-type: none"> <li>Minimize sample freeze-thaw cycles;</li> <li>Extract proteins at low temperatures in the presence of protease inhibitors;</li> <li>Load more sample.</li> </ul>
	Inefficient protein transfer.	Optimize transfer conditions: extend the transfer time, increase the voltage/current, or choose a membrane with a larger pore size.
	The blocking solution interferes with signal detection.	Use Protein-Free Blocking Buffer(1×) (Cat. No. BM3152 )
Membrane has brown or yellow bands	Too much HRP in the system. The brown bands are caused by the inactivation of HRP due to the overproduction of free radicals during substrate catalysis.	<ul style="list-style-type: none"> <li>Load less sample;</li> <li>Decrease antibody concentrations;</li> <li>Select an appropriate chemiluminescent substrate.</li> </ul>

# Choosing the right substrate

YamayBio Substrate	Cat. No.	Sensitivity	Signal Duration (hours)	Recommended Antibody Dilutions (From 1 mg/mL stock)
ECL Western Blotting Ultimate Sensitivity Substrate (Femto)	VQ4726	Low to mid femtogram	4	Primary antibody 1:10,000 Secondary antibody 1:50,000-1:100,000
ECL Western Blotting Sensitivity Substrate (Pico)	VQ4727	Low picogram to mid femtogram	4	Primary antibody 1:5,000 Secondary antibody 1:20,000-1:500,000

## Related products

Product	Cat. No.	Quantity
Stain-Free Bis-Tris Precast Gels, 8%, 12wells	AN9321-10   AN9321-20	10 Pieces /Box   20 Pieces /Box
Stain-Free Bis-Tris Precast Gels, 8%, 15wells	AN9351-10   AN9351-20	10 Pieces /Box   20 Pieces /Box
Stain-Free Bis-Tris Precast Gels, 10%, 12wells	AN9322-10   AN9322-20	10 Pieces /Box   20 Pieces /Box
Stain-Free Bis-Tris Precast Gels, 10%, 15wells	AN9352-10   AN9352-20	10 Pieces /Box   20 Pieces /Box
Stain-Free Bis-Tris Precast Gels, 12%, 12wells	AN9323-10   AN9323-20	10 Pieces /Box   20 Pieces /Box
Stain-Free Bis-Tris Precast Gels, 12%, 15wells	AN9353-10   AN9353-20	10 Pieces /Box   20 Pieces /Box
Stain-Free Bis-Tris Precast Gels, 4~12%, 12wells	AN9324-10   AN9324-20	10 Pieces /Box   20 Pieces /Box
Stain-Free Bis-Tris Precast Gels, 4~12%, 15wells	AN9354-10   AN9354-20	10 Pieces /Box   20 Pieces /Box
Stain-Free Bis-Tris Precast Gels, 4~20%, 12wells	AN9325-10   AN9325-20	10 Pieces /Box   20 Pieces /Box
Stain-Free Bis-Tris Precast Gels, 4~20%, 15wells	AN9355-10   AN9355-20	10 Pieces /Box   20 Pieces /Box