YamayBio

ECL Western Blotting Ultimate Sensitivity Substrate (Femto)

QUICK START MANUAL

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

Contents and Storage

ECL Western Blotting Ultimate Sensitivity Substrate (Femto)

VQ4726-100 mL

VQ4726-500 mL

Storage: Store at room temperature. The product is shipped at room temperature.

Introduction

ECL Western Blotting Ultimate Sensitivity Substrate (Femto) is a chemiluminescent HRP substrate designed to maximize chemiluminescent signal output 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**. Proceed directly to membrane detection after 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	• Minimize sample freeze-thaw cycles • Extract proteins at low temperatures in the presence of protease inhibitors
	Sample overload	Reduce sample load
	Poor antibody specificity	Use a more 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	Reduce antigen or antibody concentration
	Too much HRP in the system	Further dilute HRP-conjugate
	Inadequate blocking	Optimize blocking conditions
	Inappropriate blocking reagent	Test alternative blocking reagent
	Inadequate washing	Increase duration, number, or volume of washes
	Incomplete milk powder dissolution causing particulate adhesion	Ensure complete dissolution of milk powder
No reaction or weak signal	Excess HRP causing rapid substrate depletion and signal to fade quickly	Remove excess wash buffer to maintain optimal peroxidase activity
	Insufficient antigen or antibody levels	Increase antibody concentration or incubation times
	Low protein abundance or degradation	• Minimize sample freeze-thaw cycles • Extract proteins at low temperatures with protease inhibitors • Increase sample load
	Inefficient protein transfer	Optimize transfer conditions: extend the transfer time, increase the voltage/current, or choose a membrane with a larger pore size
	Blocking reagent interference 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	• Reduce sample load • Decrease antibody concentration • Select an alternative chemiluminescent substrate

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%, 12 wells	AN9321-10	10 Pieces / Box
Stain-Free Bis-Tris Precast Gels, 8%, 15 wells	AN9351-10	10 Pieces / Box
Stain-Free Bis-Tris Precast Gels, 10%, 12 wells	AN9322-10	10 Pieces / Box
Stain-Free Bis-Tris Precast Gels, 10%, 15 wells	AN9352-10	10 Pieces / Box
Stain-Free Bis-Tris Precast Gels, 12%, 12 wells	AN9323-10	10 Pieces / Box
Stain-Free Bis-Tris Precast Gels, 12%, 15 wells	AN9353-10	10 Pieces / Box
Stain-Free Bis-Tris Precast Gels, 4~12%, 12 wells	AN9324-10	10 Pieces / Box
Stain-Free Bis-Tris Precast Gels, 4~12%, 15 wells	AN9354-10	10 Pieces / Box
Stain-Free Bis-Tris Precast Gels, 4~20%, 12 wells	AN9325-10	10 Pieces / Box
Stain-Free Bis-Tris Precast Gels, 4~20%, 15 wells	AN9355-10	10 Pieces / Box