Protocol





12 OCT 2022

Catalog Number		Size	Format
CCH375-P10L	UltraScence Femto Plus Western Substrate Powder	5 L x 2	Powder

Storage Conditions

Upon receipt make the ECL powder form into liquid form. It could be stable for up to 24 months at 25°C.

Advion Interchim

Description

The UltraScence Femto Plus Western Substrate Powder, as a luminol-based enhanced chemiluminescent substrate, is sensitive and compatible with conducting immunoblots with horseradish peroxidase (HRP) – conjugated secondary antibodies. The low picogram to low femtogram detection of antigen is enabled by UltraScence Femto Plus Western Substrate' s excellent sensitivity and long signal duration. Further, its long chemiluminescent signal duration makes both digital and film-based imaging possible without any loss of the signal. Appropriate primary and secondary antibody dilutions are suggested for attaining optimal signal intensity and duration.

- Ready-to-ship and significantly longer shelf life in the powder form!
- Significant reductions in transportation and storage costs, resulting in less carbon footprint!
- Empower custom production anywhere in the world!
- Significantly more sensitive than leading manufacturers on the market

Kit Content(s)

Catalog Number	Size	Format
CCH375-P05LA	5 L x 1	Powder/ bag
CCH375-P05LB	5 L x 1	Powder/ bag

Required materials but not provided

- A compatible Chemiluminescence or X-ray Imaging Systems
- A plastic sheet protector or plastic wrap to prevent the membrane from drying

Instrument Compatibility

This western substrate is compatible with the majority of commercially available Chemiluminescence and X-ray Imaging Systems.



Protocol



Powder dissolution

- 1. Prepare two 5L bottles. One amber bottle is for luminol solution, and other normal bottle is for peroxide solution. (Make sure the bottles are acid and alkali resistant.)
- 2. Unpack the bag and pour all the powder in bottle.
- 3. Rinse residual luminol powder in bag with 10% (v/v) ethanol solution. Add 10% (v/v) ethanol solution to 5L for luminol solution.
- 4. Rinse residual peroxide powder in bag with deionized water. Add deionized water to 5 L for peroxide solution.

Reaction Setup

- 1. Keep the membrane moist in the wash buffer while preparing the substrate mixture. Please ensure the membrane does not dry out during the subsequent steps.
- 2. Mix Luminol solution and Peroxide Solution in a 1:1 ratio, and thoroughly agitate the chemiluminescent substrate solution well for preparing the 0.1 ml of solution / cm² of membrane.
 - For a mini-sized membrane (7 x 8.5 cm), 4 ml of solution is sufficient.
 - For a midi-sized membrane (8.5 x 13.5 cm), 10 ml of solution is sufficient.
- 3. Place the membrane with the protein side up on a clear and level surface or in a clean container.
- 4. Remove the membrane from the chemiluminescent substrate solution and drain off excessive solution.
- 5. Place the membrane in a plastic sheet protector or in plastic wrap to prevent the membrane from drying.
- 6. Image the membrane with a digital imager or by exposing to the X-ray film.







Protocol





Important notes

UltraScence ECL substrates series is compatible with the use from low picogram to low-femtogram level detections. Please kindly refer to the ECL selection guide of UltraScence Western substrate as the below table.

Bio-Helix Western Substrates	Advantages for you	Sensitivity	Compare Performance to
UltraScence <u>Pico Plus</u> Western Substrate CCH321-B100ML	Best value of abundant protein detection and best sensitivity among entry-level western substrate	low picogram or high femtogram	-Thermo Scientific [™] Pierce ECL Substrate -Thermo Scientific [™] SuperSignal [™] West Pico PLUS -CYANAGEN WESTAR NOVA 2.0 -Advansta WesternBright [™] ECL -Abcam High Sensitivity ECL Substrate Kit
UltraScence <u>Pico Ultra</u> Western Substrate CCH345-B100ML	Better choice when seeking low abundance proteins, over 30 times sensitivity than UltraScence Pico Plus western substrate.	low picogram to mid femtogram	-Millipore TM Immobilon TM Western Substrate -FUJIFILM Wako ImmunoStar Zeta -Cytiva Amersham ECL Prime -Advansta TM WesternBright TM Quantum TM -Abcam Very High Sensitivity ECL Substrate Kit -CYANAGEN WESTAR ETA C ULTRA 2.0 -Thermo Scientific TM SuperSignal TM West DURA
UltraScence <u>Femto Plus</u> Western Substrate CCH375-B100ML	Born to seek , seeking less abundance proteins in your Western Blot, even low femtograms.	mid femtogram to low femtogram	-FUJIFILM Wako ImmunoStar LD -GeneTex Trident femto -Thermo Scientific TM SuperSignal TM West Femto -Advansta TM WesternBright TM Sirius TM -Abcam Ultra High Sensitivity -CYANAGEN WESTAR SUPERNOVA -Cytiva Amersham TM ECL Select TM
UltraScence <u>Atto</u> Western Substrate CCH385-B100ML	Break the record , providing the most sensitive and brightest protein signal for your Western Blot.	Low femtogram to high attogram	-CYANAGEN WESTAR HYPERNOVA -Thermo Scientific™ SuperSignal™ West Atto

Troubleshooting

Problem	Cause	Solution	
High Background	Overconcentrated primary	*Decrease the antibody concentration.	
	or secondary antibody	*Perform a dot blot to optimize the concentration.	
	Insufficient wash	*Increase the frequency or duration.	
	Incomplete blocking	*Decrease the antibody concentration.	
		*Perform a dot blot to optimize the concentration.	
No Reaction or Weak	Insufficient antigen	*Decrease antibody concentration.	
Signal	binding	*Optimize blocking reagents for achieving a	
		balance between sensitivity and specificity.	
	Poor antibody binding to	*Optimize detergent used for antibodies.	
	the antigen	*Increase the antibody incubation time.	
No Reaction or Weak	Proteins washed from the	*Reduce the number or intensity of wash	
Signal	membrane during assay		
	Insufficient reagent	*Apply additional volumes of antibody blocking	
	volume	reagent, or wash solution.	

5F., No. 145, Sec. 3, Beixin Rd., Xindian Dist., New Taipei City, Taiwan (R.O.C.) TEL : +886-2-2462-4956 / FAX : +886-2-2462-8849 www.bio-helix.com

BIO-HE