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Datasheet for FEMTOMAX-020

Chemiluminescent FemtoMax™ Super Sensitive HRP Substrate

Overview

Description:	Chemiluminescent FemtoMax™ Super Sensitive HRP Substrate for Microwell and/or Membrane (2 component system) - FEMTOMAX-020
Item No.:	FEMTOMAX-020
Size:	20 mL
Applications:	WB, ELISA

Product Details

Background:	FemtoMax™ Super Sensitive Chemiluminescent HRP Substrate is an extremely sensitive, nonradioactive, enhanced luminol-based chemiluminescent substrate for the detection of horseradish peroxidase (HRP). FemtoMax™ is designed for both Western blotting and enzyme-linked immunosorbent assay (ELISA) use. FemtoMax™ easily allows for the detection of femtogram (10-15) amounts of antigen using photographic film or other imaging methods, including highly sensitive CCD cameras. Blots can be repeatedly exposed to X-ray film to obtain optimal results or stripped of detection reagents and re-probed. Use the same blotting conditions for FemtoMax™ as you would when using Amersham ECL Plus™ Substrate or Pierce SuperSignal® West Femto Substrate.
Synonyms:	Peroxidase Substrate, ECL HRP Substrate, Chemiluminescent Femtomax™ Super Sensitive horseradish peroxidase (HRP) Substrate For Microwell And/Or Membrane (2 Component System)

Target Details

Relevant Links:

- FemtoMax SDS

Application Details

Tested Applications:	WB
Suggested Applications:	ELISA (Based on references)

Application Note:	Prepare FemtoMax™ Super Sensitive Chemiluminescent HRP Substrate for use in microwell or membrane applications by mixing 1 mL of Luminol Reagent (Reagent A) with 1 mL of Reaction Buffer (Reagent B). Mix well. Protect from light. Larger or smaller volumes of the substrate can be prepared by mixing components at the same 1:1 ratio. FemtoMax™ Super Sensitive Chemiluminescent HRP Substrate is a highly sensitive detection reagent. Always carefully optimize all components of individual assays (antigens, antibodies, conjugates…) to minimize background reactivity associated with non-specific binding.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1X
WB:	1X

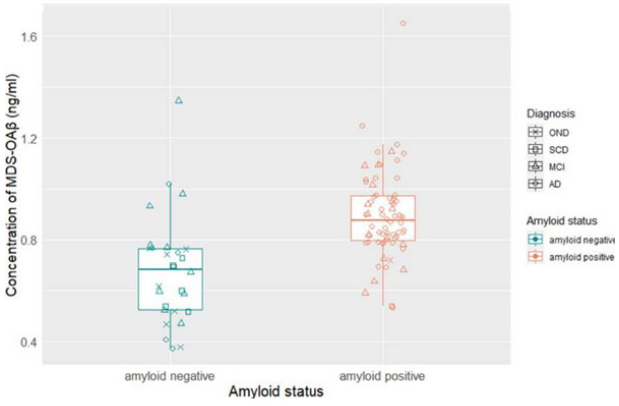
Formulation

Physical State:	Liquid - clear, colorless, odorless

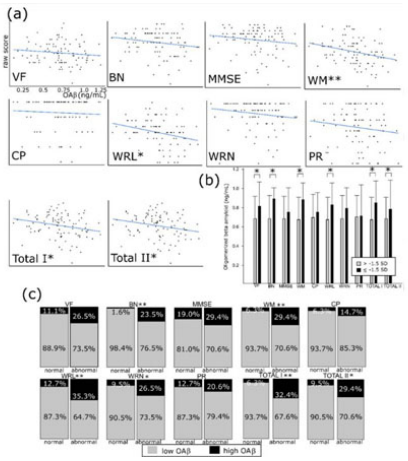
Shipping & Handling

Shipping Condition:	Wet Ice
Storage Condition:	Store container at 4° C prior to opening. Protect from moisture and light. No special shipping conditions or precautions are required.

Images



ELISA
Concentration of plasma MDS-OAβ according to groups. The incubated plasma sample mixture and serially diluted standard samples were added to respective wells, and the plates were incubated at room temperature for 1 hour. Afterwards, 100 µL/well of enhanced chemiluminescence substrate solution (p/n Femtomax) was added, and the Relative Luminescence Unit (RLU) signal was detected using a Victor 3TM multi-spectrophotometer.
Abbreviations: AD, Alzheimer's disease; MCI, mild cognitive impairment; MDS-OAβ, Multimer Detection System-Oligomeric Amyloid-β; OND, other neurodegenerative disease; SCD, subjective cognitive decline.
Fig 1. PMID: 33958861.

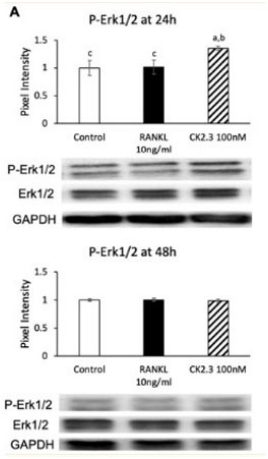


ELISA

The subtests of the Korean version of Consortium to Establish a Registry for Alzheimer's disease (CERAD-K) and plasma oligomerized beta amyloid (OAβ). (a) Raw scores of the CERAD-K and OAβ concentration. (b) The abnormal CERAD group (below −1.5 standard deviation of age/sex/education adjusting norms) in verbal fluency, naming, word memory/recall, and total scores showed higher OAβ concentration compared with control. (c) Abnormality in naming, word memory/recall/recognition, and total scores are significantly more frequent in high OAβ groups (≥ 0.78 ng/mL). SD—standard deviation; VF—verbal fluency; BN—Boston naming; MMSE—mini-mental status examination; WM—word list memory; CP—constructional praxis; WRL—word list recall; WRN—word list recognition; and PR—praxis recall. Total I is a sum of subtests except MMSE and PR and Total II is a sum except MMSE. * $p < 0.05$. ** $p < 0.01$.

To increase the sensitivity of detection, 100 µL/well of enhanced chemiluminescence substrate solution (p/n Femtomax) was added, and the Relative Luminescence Unit (RLU) signal was detected using a Victor 3TM multi-spectrophotometer.

Fig 2. PMID: 32326061.



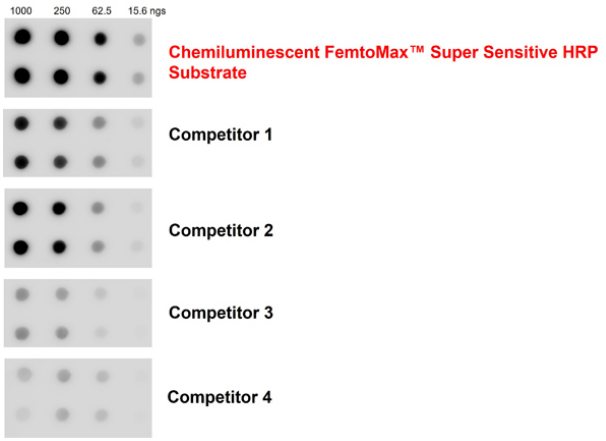
Western Blot

Effect of CK2.3 on p-Erk 1/2 in RANKL-induced osteoclastogenesis. CK2.3 increased p-Erk 1/2 after 24 h of stimulation in RAW264.7 cells, as determined by (A) Western blotting.

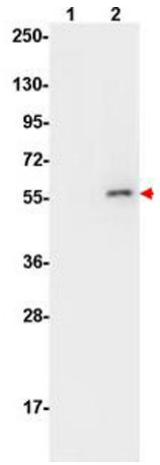
The blot was incubated in 3% BSA for 1 h to block non-specific binding. Antibodies used at 1:1000 dilutions (in 1% BSA) overnight at 4 °C. Followed by incubation with the secondary antibody HRP anti-rabbit at a 1:5000 dilution (in 1% BSA). The blot was incubated in Chemiluminescent FemtoMax Super Sensitive HRP Substrate (p/n Femtomax) for 2 min. Fig 6. PMID: 32660129.



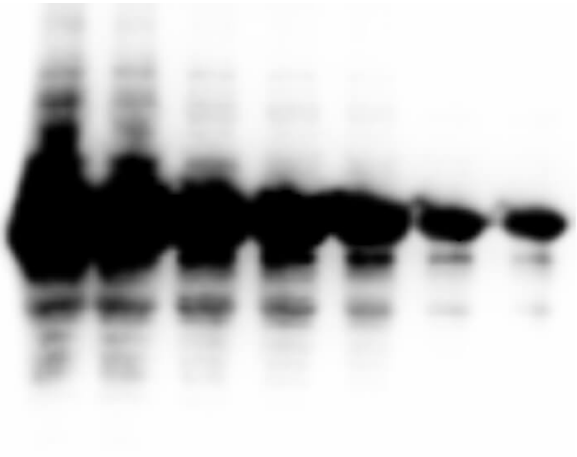
Bottle
Chemiluminescent FemtoMax™ Super Sensitive HRP Substrate for Microwell and/or Membrane (2 component system)



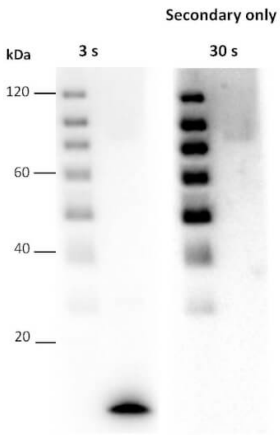
Dot Blot
Chemiluminescent FemtoMax™ Super Sensitive HRP Substrate and competitor comparison.
GAPDH protein was dotted on a nitrocellulose membrane, load 1000, 250, 62.5, 15.6ngs. The membrane was blocked for one hour at room temperature. Primary antibody: Rb anti-GAPDH diluted 1:2000 and incubated at 4°C overnight. After washing, secondary antibody: Gt anti-Rb IgG-HRP diluted 1:50,000 at room temperature for 2 hours. Detection: Chemiluminescent FemtoMax™ Super Sensitive HRP Substrate or competitor 1-4.



Western Blot
Western Blot of Mouse Anti-AKT pS473 antibody using Femtomax.
Lane 1: non-phosphorylated AKT in untreated cells.
Lane 2: phosphorylated AKT (indicated by arrowhead at ~56 kDa) on PDGF stimulated NIH/3T3 cell lysates.
Load: 10 µg per lane.
Primary antibody: AKT pS473 antibody at 1:10,000 in TBS with 0.05% Tween-20 with 1% BSA, for 1 h at 4° C.
Secondary antibody: HRP conjugated Gt-a-Mouse IgG (p/n 610-103-121) was used at a 1:20,000 dilution for 1 h at 4° C with Chemiluminescent FemtoMax™ Super Sensitive HRP Substrate (p/n FEMTOMAX-100).



Western Blot
Western Blot of anti-GST tag antibody. Lane 1: Recombinant GST tagged recombinant protein 5 ug. Lane 2: Recombinant GST tagged recombinant protein 2 ug. Lane 3: Recombinant GST tagged recombinant protein 1 ug. Lane 4: Recombinant GST tagged recombinant protein 500 ng. Lane 5: Recombinant GST tagged recombinant protein 250 ng. Lane 6: Recombinant GST tagged recombinant protein 100 ng. Lane 7: Recombinant GST tagged recombinant protein 50 ng. Primary antibody: anti-GST antibody at 1:1000 for overnight at 4°C. Secondary antibody: donkey secondary antibody at 1:10,000 for 45 min at RT. Block: 5% BLOTTO overnight at 4°C. Predicted/Observed size: 78 kDa.



Western Blot
Western blot detection using Chemiluminescent FemtoMax™ HRP Substrate. rPARP1 domain detected at 11 kDa after 3 sec exposure using primary antibody rabbit anti-serum 1:500 overnight, at 4°C. Secondary antibody Peroxidase Goat Anti-Rabbit IgG Antibody at 1:40,000. All incubations were performed in Blocking Buffer for Fluorescent Western Blocking (p/n MB-070).

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