Cat. # T9320A

For Research Use

TaKaRa

TaKaRa CBB Protein Safe Stain

Product Manual

v201510Da

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I. Description

TaKaRa CBB Protein Safe Stain is a highly sensitive protein staining reagent based on Coomassie Brilliant Blue G-250. This product can be used to stain SDS-PAGE or native PAGE gels quickly. It allows the visualization of protein bands approximately 5 minutes after the staining process begins, and provides maximum sensitivity after 60 minutes of staining. Stained gels may be destained with deionized water to remove background staining. TaKaRa CBB Protein Safe Stain can detect bands containing as little as 8 ng of protein. It can be handled safely, since it does not contain methanol and acetic acid.

II. Components

TaKaRa CBB Protein Safe Stain 1 L

III. Storage

Room temperature

IV. Precautions for Use

Read these precautions before using the product.

1. Before use, gently shake or turn the bottle upside down until the solution is thoroughly mixed. Avoid vigorous shaking.
2. This product is slightly corrosive, since it is a weak acid. Always wear a lab coat, safety goggles, and gloves when using this product.
3. When performing the quick protocol, which uses a microwave oven, make sure to avoid overheating, which may cause the solution to boil violently and the gel to break. As the heated solution is very hot, be careful to avoid burning yourself.
4. The protocols assume a gel thickness of 1 mm. If the gel thickness differs substantially, adjust the solution volume and staining time as appropriate.

V. Protocol

Protein electrophoresis and gel staining

1. Standard protocol (required time: 140 minutes)

• Gel washing After electrophoresis, wash the gel with deionized water.

SDS-PAGE gel: Transfer the gel to an appropriate tray. If you are using mini gels, perform three 5-minute washes, each with 50 ml of deionized water.

Native PAGE gel: Transfer the gel to an appropriate tray. If you are using mini gels, perform one 5-minute wash with 50 ml of deionized water.

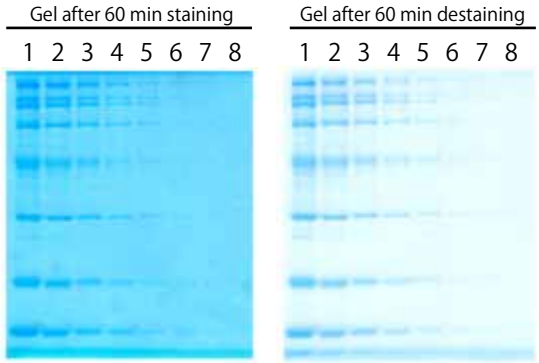
• Staining

After mixing TaKaRa CBB Protein Safe Stain thoroughly, add 25 - 50 ml of the product to an appropriate tray. Use enough of the product to completely immerse the gel while it is shaking, usually 25 ml for mini gels. Completely immerse the washed gel, and shake gently for 30 - 60 minutes.

In general, bands with 15 - 20 ng of protein are visible after 30 minutes of staining. Maximum sensitivity, with a detection limit of 8 ng of protein, is achieved after 60 minutes. Perform staining at room temperature (about 25℃), since sensitivity may decrease at ≦15℃.

• Destaining Perform destaining as needed to remove background staining.

Discard the staining solution and wash the gel gently with deionized water. Then destain the gel by washing twice in either 100 ml of deionized water for 60 minutes or 50 ml of deionized water for 30 minutes with shaking. Repeat the destaining procedure once.



1:	Protein MW Marker (Broad)	1,000 ng/lane
2:	Protein MW Marker (Broad)	500 ng/lane
3:	Protein MW Marker (Broad)	250 ng/lane
4:	Protein MW Marker (Broad)	125 ng/lane
5:	Protein MW Marker (Broad)	62.5 ng/lane
6:	Protein MW Marker（Broad）	31.3 ng/lane
7:	Protein MW Marker（Broad）	15.6 ng/lane
8:	Protein MW Marker（Broad）	7.8 ng/lane

Figure 1. Stained 12% SDS-PAGE gel.

2. Quick protocol (required time: 40 minutes)

This protocol uses a microwave to heat the staining and destaining solutions to a high temperature, which substantially reduces the time required.

• Gel washing After electrophoresis, wash the gel with deionized water.

SDS-PAGE gel: Transfer the gel to an appropriate tray. If you are using mini gels, perform three 5-minute washes, each with 50 ml of deionized water.

Native PAGE gel: Transfer the gel to an appropriate tray. If you are using mini gels, perform one 5-minute wash with 50 ml of deionized water.

• Staining

After mixing TaKaRa CBB Protein Safe Stain thoroughly, add 25 - 50 ml of the product to a microwave-safe tray. Use enough of the product to completely immerse the gel, usually 25 ml for mini gels. Completely immerse the washed gel in the solution.
Heat the tray in a microwave oven until the staining solution begins to boil slightly (approximately 1 minute), then immediately stop heating. The heating time can vary, depending on the microwave oven used.

Note: Avoid overheating, since this may cause the solution to boil violently and the gel to break.

Remove the tray from the microwave oven and shake gently for 5 minutes to stain the gel. The quick protocol is as sensitive as staining for 60 minutes at room temperature, allowing the detection of bands containing as little as 8 ng of protein.

• Destaining

Perform destaining, as appropriate, to remove background staining.Discard the staining solution and wash the gel gently with deionized water. Exercise caution, since the gel and tray are hot. Add 200 ml of deionized water (heated to about 90℃) to the tray and shake gently for 10 minutes to destain the gel. Never destain for as long as 15 minutes or more. You may use water (tap or potable) boiled on an electric pot or a similar device, or hot water that has been maintained at about 90℃.

Membrane (PVDF/nitrocellulose) staining

This protocol provides procedures for staining membranes onto which proteins have been transferred by an appropriate procedure. Both PVDF and nitrocellulose membranes can be stained using this protocol, but membranes that have been treated with a blocking reagent are not suitable. A destaining solution (50% methanol, 1% acetic acid) is required for destaining.

• Membrane washing

Transfer the membrane to an appropriate tray and wash with deionized water for 2 minutes.

• Staining

After mixing TaKaRa CBB Protein Safe Stain thoroughly, add 25 - 50 ml to an appropriate tray. Use enough of the product to completelly immerse the membrane while it is shaking, usually 25 ml for mini gel-sized membranes. Shake gently for 5 minutes to stain.

• Destaining

Prepare the destaining solution (50% methanol, 1% acetic acid), and discard the staining solution. Destain for 5 minutes, using the same volume of destaining solution as staining solution. Repeat the destaining 2 to 3 times.

VI. Related Products

Tris-Glycine-SDS Buffer (TG-SDS) Powder, pH 8.3 (Cat. #T9101)
Tris-Glycine Buffer (TG) Powder, pH8.3 (Cat. #T9102)
CLEARLY Protein Ladder (Unstained) (Cat. #3453A/B)
CLEARLY Stained Protein Ladder (Cat. #3454A/B)
TaKaRa BCA Protein Assay Kit (Cat. #T9300A)
TaKaRa Bradford Protein Assay Kit (Cat. #T9310A)