Wako Product Update

Protein Research
Cell Culture

Please visit the Wako Online Catalog
http://search.wako-chem.com

Wako

No. 10

WakoPURE system
Quick-CBB PLUS
Silver Stain MS Kit
Negative Gel Stain MS Kit
Matrix for MALDI-TOFMS
BAMBANKER

Protein Research
Cell Culture

2004

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ALPHABETICAL INDEX

Next-Generation in vitro Protein-synthesizing System

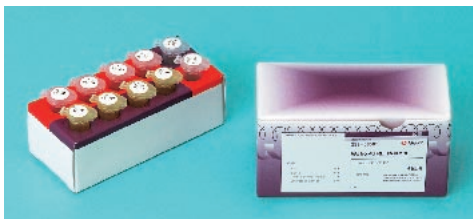
WakoPURE system

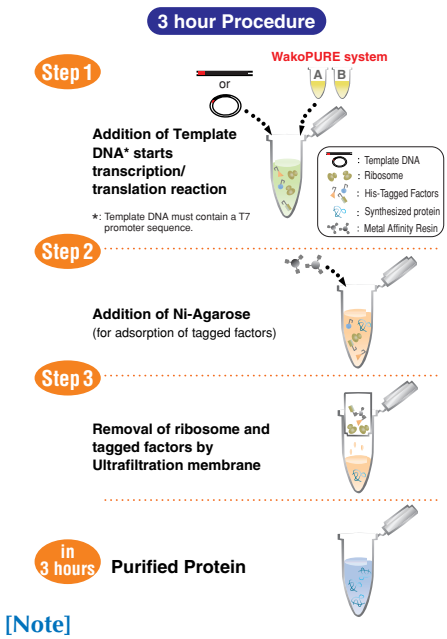
Individual Components bring out the Maximum Performance !

WakoPURE system is a proprietary protein synthesis & purification technology, which is an *in vitro* protein synthesizing system "reconstituted" from translation factors expressed in *Escherichia coli*.
It is a novel reconstituted system consisting of around 30 purified enzymes necessary for transcription, translation and energy recycling.
All the components involved in transcription and translation except for ribosomes are tagged with hexahistidine at the N or C terminus. Because all the factors are tagged and known components, synthesized protein can be easily purified by simply removing both the ribosome by ultrafiltration and the histidine-tagged factors and enzymes by metal affinity resin. protein of interest can be synthesized and purified about 3 hours.

[Features]

- **No Tag Required**
  Because all the transcription/translation factors are tagged, synthesized protein can be easily purified. Native form, without any tags.
- **Time Saving**
  All you need is to only 3 steps; synthesis, affinity chromatography and ultrafiltration. It takes only 1 minute for handling and 2 hours for incubation and purification.
- **Pure Protein Synthesis System**
  WakoPURE system little contain contaminants such as protease and nuclease because of a novel reconstituted system.
- **Up to 100 Ug protein per 1 mL reaction**
  DHFR (dihydrofolate reductase) can be synthesized at a yield of 50 μg/mL in 1 hour.





[Note]
Plasmid DNA and PCR product can be used with WakoPURE system as a template DNA, and the template must contain the following components:
An initiation codon (ATG)/A stop codon (TAG, TGA or TAA)/ A T7 promoter sequence located upstream of the target gene/ Prokaryotic Shine-Dalgarno ribosome binding site (SD sequence) located 10 nt upstream of the initiation codon./ More than 6 nt must have at the upstream of the stop codon for the PCR product. No terminator sequence is necessary./ A T7 terminator located in the downstream of the stop codon for plasmid DNA.

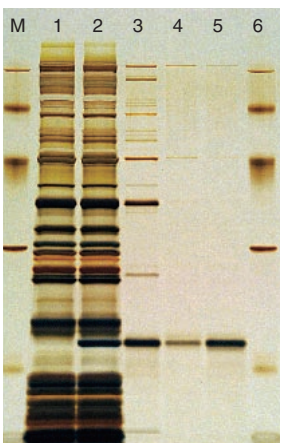


Figure
M, 6 : Molecular Weight Marker
1 : Negative Control (no DNA template)
2 : Positive Control (DHFR control plasmid) at Step 1
3 : Sample obtained at Step 1 was purified by ultrafiltration with WakoPURE MF-100K (Wako Cat. #237-02231)
4 : Sample obtained at Step 1 was treated by Ni-Agarose (Wako Cat.#145-07981) (removal of His-tagged factors) and filtered by WakoPURE Spin Empty Column (Wako Cat. #234-02241).
5 : Sample obtained at Step 1 was treated by Ni-Agarose and purified by ultrafiltration with WakoPURE MF-100K

Result of DHFR after synthesis and various purification. (Apply samples on a 12.5% SDS-PAGE gel and electrophoresed, followed by staining the gel with Silver Stain II Kit Wako (Wako Cat. #291-50301)

Catalog No.	Description	Kit contents	Package Size
299-59501	WakoPURE system	Solution A/ Solution B/DHFR control vector (positive control)/ Universal Primer	4 reactions
295-59503			16 reactions

This kit provides the necessary for procedures until protein synthesis at the step 1.

Related Products

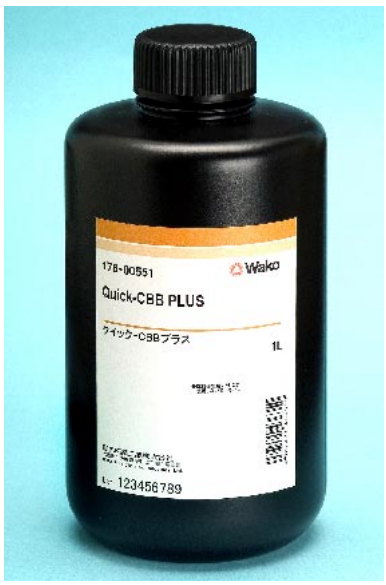
Catalog No. (Package Size)	Description		
145-07981 (5mL), 141-07983 (10mL), 149-07984 (100mL)	Ni-Agarose		Available Soon
237-02231 (20 EA)	WakoPURE MF-100K	ultrafiltration membrane	
234-02241 (20 EA)	WakoPURE Spin Empty Column	filtration column	

Quick-CBB PLUS

for electrophoresis

Cat.# 174-00553 250 mL
178-00551 1 L

Quick-CBB PLUS is a bottled protein staining kit, which allows simple and quick staining of proteins bands in polyacrylamide slab gels. In comparison with conventional Quick-CBB, fixing procedure is not required and organic solvents such as methanol and acetic acid are not used. The mixing procedure is now also unnecessary as all the required solutions for staining are contained in a single bottle. There are also other improvements such as coloration not occurring on the background. As in conventional Quick-CBB, destaining procedure is not required.



[Features]
- **No fixing procedure and no organic solvents are necessary.**
- **Simple and quick staining without the coloration on background**

Quick-CBB PLUS Staining Protocol

1. Washing
Wash gel 2 times with deionized water
5 minutes × 2 times

→

2. Staining
Stain gel by adding Quick-CBB PLUS
Shake for 30 ~ 60 min.

→

3. Washing
Wash gel for 1 hour with deionized water
Shake for 60 min.

→

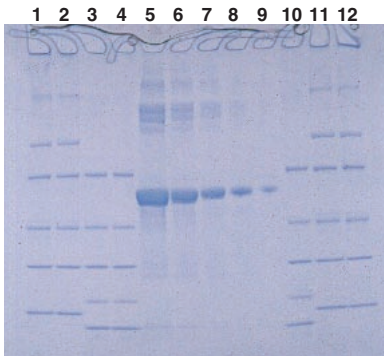


Figure : Quick-CBB PLUS Stain
using 5~12% gel
Sample: Lane 1, 2, 11 and 12 : Molecular Weight Marker, High Range (Wako Cat.#134-14501);
Lane 3,4 and 10 : Molecular Weight Marker, Middle Range (Wako Cat.#131-14511)
Lane 5, 6, 7, 8 and 9 : 10, 5, 2.5, 1.25 and 0.6μg of BSA, respectively

FAQ

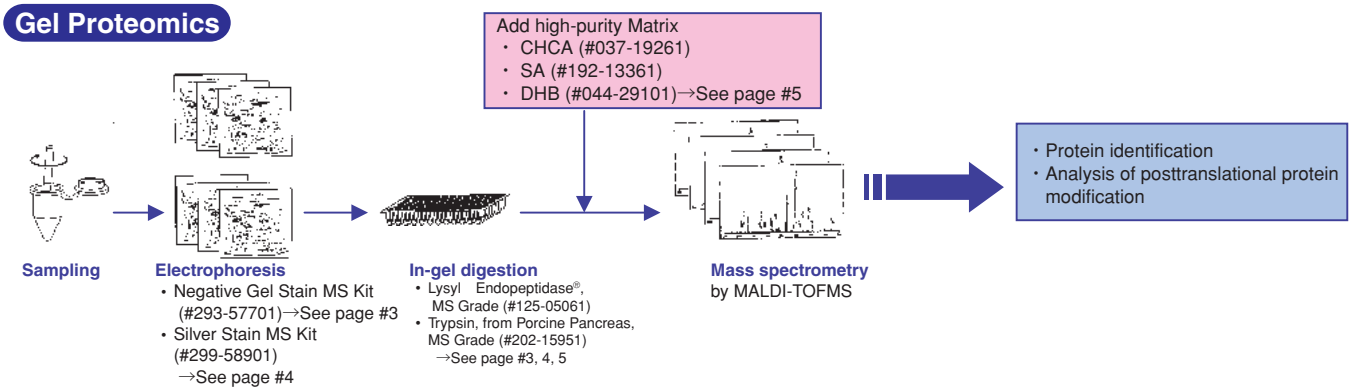
Q1 : When protein bands are detected a few minutes after the start of staining, can the staining procedure be terminated by washing the gel?
A1 : Quick-CBB PLUS usually stains protein bands in 10-20 minutes. The staining can be terminated at that time, but 30-60 minutes is recommended for proper staining.
Q2 : When protein bands can be clearly detected and background staining does not occur without washing after staining, is the washing procedure still required?
A2 : The washing procedure is not imperative, but a clearer staining can be obtained by washing the gel. When protein bands are light after 60-minute of washing, a clearer staining can be obtained after overnight washing.
Q3 : About disposal
A3 : Quick-CBB PLUS does not contain any harmful substances, but is a very dark blue color. Therefore it should be collected in a container prepared for disposal.
Q4 : As for the sensitivity of the staining
A4 : Some protein bands visible to approx. 10 ng

Catalog No.	Description	Grade	Package Size	Storage
174-00553	Quick-CBB PLUS	for electrophoresis	250 mL	RT
178-00551			1 L	

Related Products

Catalog No.	Description	Grade	Package Size	Storage
134-14501	Molecular Weight Marker, High Range	for electrophoresis	1 mL	2 ~ 10℃
131-14511	Molecular Weight Marker, Middle Range		1 mL	

Gel Proteomics



a. Silver Stain MS Kit

Silver Stain MS Kit

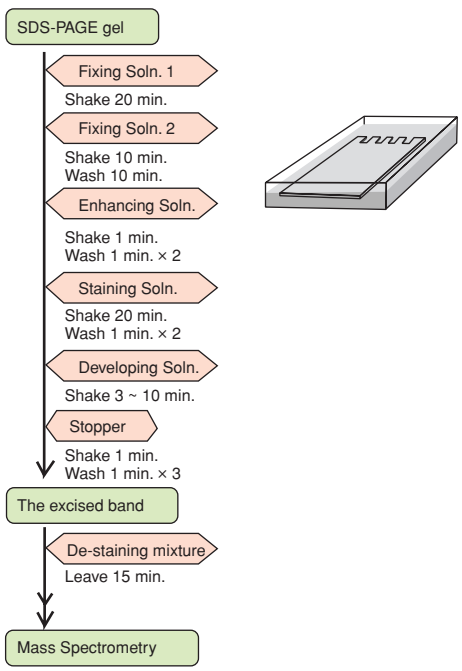
Cat. # 299-58901 20 tests

This silver staining kit is produced for mass spectrometric sequencing of protein separated by polyacrylamide gel electrophoresis, based on the method described by Shevchenko *et al*. By silver staining, protein is rarely modified chemically due to omitting treatment of glutaraldehyde and is detected at sub-nanogram level on the electrophoretic gel.

[Features]

- **Detectable limit of protein : 1 ng**
- **Ideal for MS analysis because rarely modified protein is obtained due to omitting glutaraldehyde treatment.**

Procedure



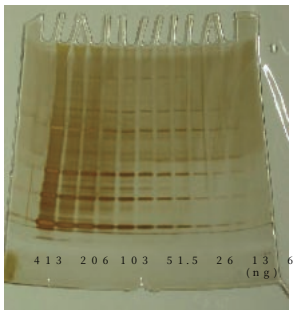


Figure 1. SDS-PAGE analysis using Silver Stain MS Kit

Samples were molecular weight markers, containing myosin, β-D-galactosidase, BSA, aldolase, carbonic anhydrase and myoglobin

[Kit Contents]

1. Enhancing Stock Soln.	1 × 200 mL
2. Staining Stock Soln.	1 × 200 mL
3. Developing Stock Soln.	1 × 100 mL
4. Developing Powder	1 × 20 g
5. Stopper	1 × 200 mL
6. De-staining Soln. A	1 × 50 mL
7. De-staining Soln. B	1 × 50 mL

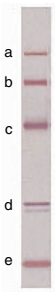


Figure 2.
SDS-PAGE analysis of proteins using Silver stain MS Kit

a: rat phosphorylase (97k);
b: bovine serum albumin (66k);
c: hen egg white ovalbumin (45k);
d: bovine carbonic anhydrase (31k);
e: soybean trypsin inhibitor (21k)
(100 ng each)

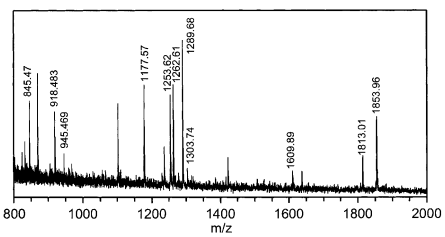


Figure 3. MALDI-TOF/MS of rabbit phosphorylase

The band was excised and treated with Lysyl Endopeptidase® (#125-02543). Following the in-gel digestion and preparation, the sample was analyzed on MALDI-TOF mass spectrometer.
(These data were provided by Dr. Y. Wada at Osaka Medical Center, Japan.)

[Reference]

1. Shevchenko, A., *et al., Anal.Chem.*, **68**, 850 (1996)
2. Farzin, G., *et al., Electrophoresis*, **20**, 601 (1999)

Catalog No.	Description	Grade	Package Size	Storage
299-58901	**Silver Stain MS Kit**	for electrophoresis	20 tests	2 ~ 10℃

b. Negative Gel Staing MS Kit

Negative Gel Staing MS Kit

Cat. # 293-57701

It is known that protein bands separated by SDS/polyacrylamide gel electrophoresis (SDS-PAGE) can be visualized as transparent bands against the background of milky white gel stained by negative gel stain containing a Zn/imidazol reagent. We have improved the method using a new imidazol derivative reagent, which allows a clear and stable image of protein bands on the gel as sensitive as that by silver staining, in as little as 10 minutes. The staining technique is useful to obtain the clear and sensitive resolution pattern of the gel before immunoblotting as well as to excise and purify the band of interest from the gel without significant deterioration of amino acid residues for the subsequent studies of protein such as sequencing and mass analysis of peptide.

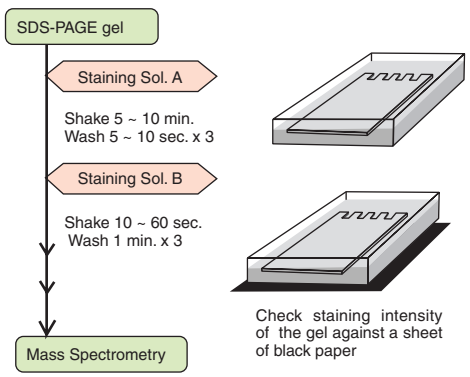
[Features]

- **Detectable limit of protein: 1 ng**
- **Sensitive and Simple procedure with 2-step staining in 5 ~ 10 minutes**
- **Optimized the staining intensity by either de-staining the excess stain or re-staining following de-stain of the gel.**
- **Applicable to proteome analysis by mass spectrometry and sequencing**

[Kit Contents]

1.	Staining Solution A	1 x 500 mL
2.	Staining Solution B	1 x 500 mL
3.	De-staining Solution	1 x 500 mL

Procedure



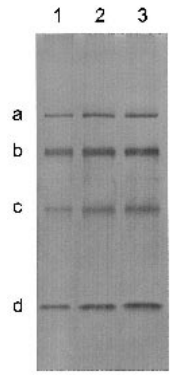


Figure 1.
SDS-PAGE analysis of proteins using Negative Gel Stain MS Kit

a : rat phosphorylase (97k);
b : bovine serum albumin (68k);
c : hen egg white ovalbumin (45k);
d : bovine carbonic anhydrase (31k).
Lane 1: 50 ng; Lane 2: 100 ng; Lane 3: 150 ng

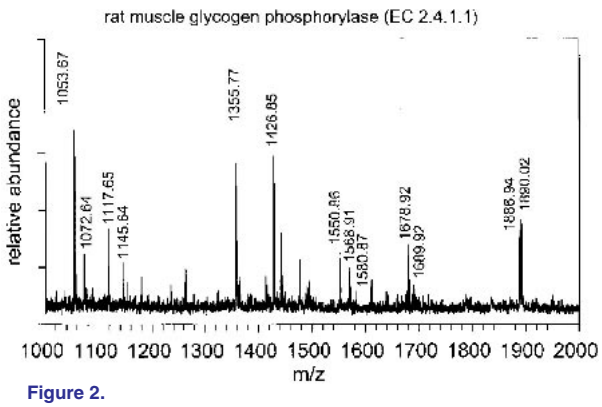


Figure 2.
MALDI-TOF/MS of rat phosphorylase

The band was excised and treated with trypsin. Following the in-gel digestion and preparation, the sample was analyzed on MALDI-TOF mass spectrometer.

(These data were provided by Dr. Y. Wada at Osaka Medical Center, Japan.)

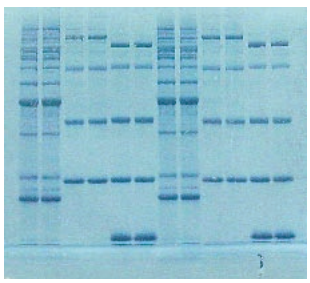


Figure 3. SDS-PAGE analysis of MW markers using Negative Gel Stain MS Kit run on 10% gel.

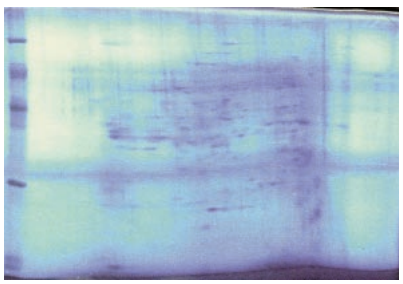


Figure 4. Negative Gel Stain MS Kit-stained 2-D gel.

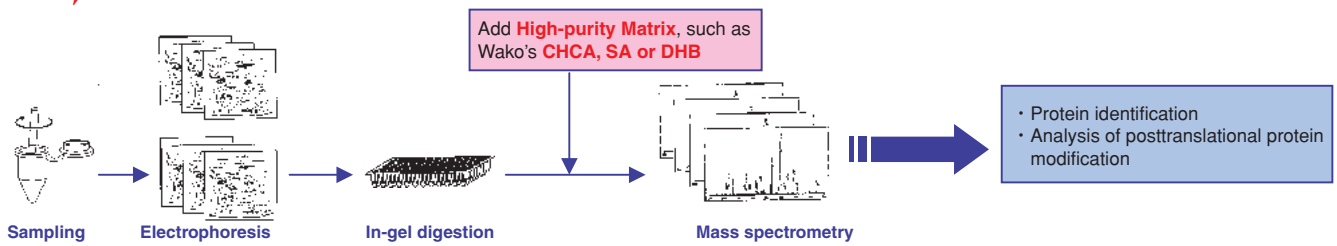
Pollen tube protein was applied to a dry strip 3-10. The second dimension was performed on a 12% gel.

[Reference] Fernandez-patron, *et al., Anal. Biochem.*, **224**, 263 (1995)

Catalog No.	Description	Grade	Package Size	Storage
293-57701	**Negative Gel Stain MS Kit**	for electrophoresis	20 tests	Room Temperature

c. High-purity Matrix for MALDI-TOFMS analysis

CHCA, SA and DHB

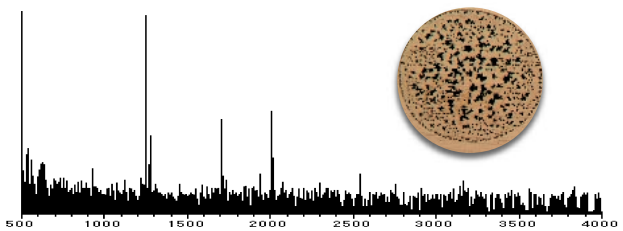


[Features]

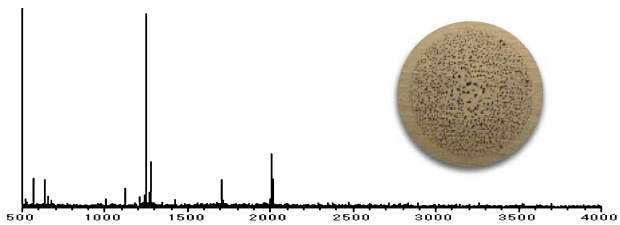
- **High purity matrix is mixed with a test sample and used for proteome analysis by mass spectrometry (MALDI-TOFMS).**
- **High purity matrix gives good mass spec reading because of its high purity by recrystallization**

Effects of recrystallization of α-Cyano-4-hydroxycinnamic Acid (CHCA)

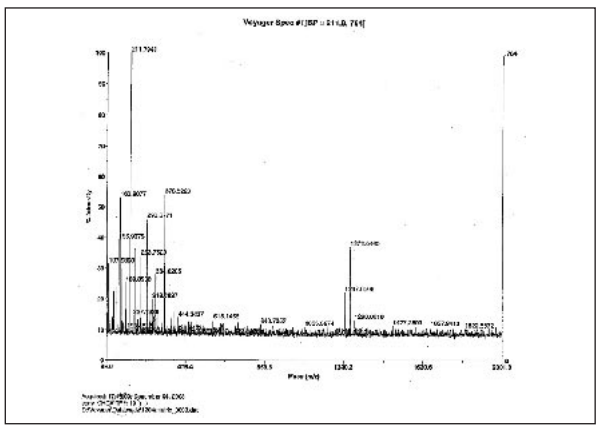
When comparing with MALDI-TOFMS data for commercial CHCA, it was found that recrystallized CHCA (Wako Catalog No. 037-19261) gives clearer mass spec reading with less background noise. (These data were provided by Dr. Wada Y. at Osaka Medical Center, Japan)



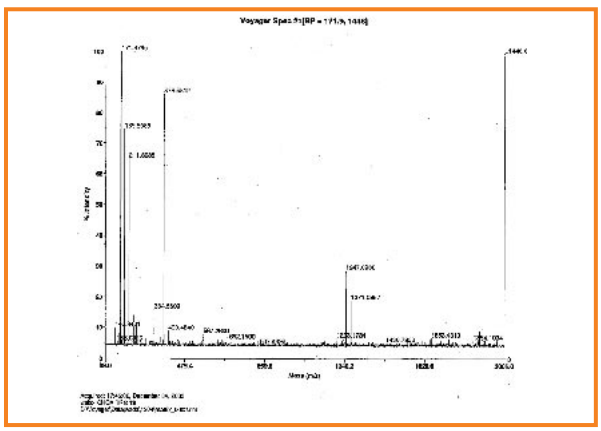
Untreated CHCA



Recrystallized CHCA



Untreated CHCA with a peptide sample



Recrystallized CHCA with the peptide sample

[Product List]

Catalog No.	Product	Grade	Package Size	Storage
037-19261	α-Cyano-4-hydroxycinnamic Acid [CHCA]	for proteome research	5 × 50 mg	2~10℃
192-13361	Sinapic Acid [SA]		5 × 50 mg	
044-29101	2,5-Dihydroxybenzoic Acid [DHB]		5 × 50 mg	

Related Products

Catalog No.	Product	Grade	Package Size	Storage
125-05061	Lysyl Endopeptidase®, Mass Spectrometry Grade	for proteome research	5 × 20 μg	-20℃
202-15951	Trypsin, from Porcine Pancreas, Mass Spectrometry Grade		5 × 20 μg	

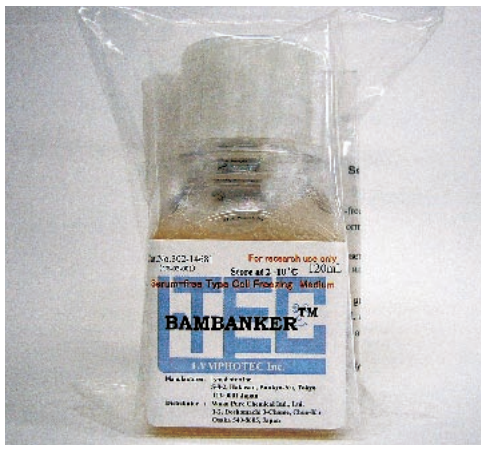
Serum-Free Cell Freezing Medium

BAMBANKER™

Cat. #302-14681 (120 mL)

Keep at 2 ~ 10℃

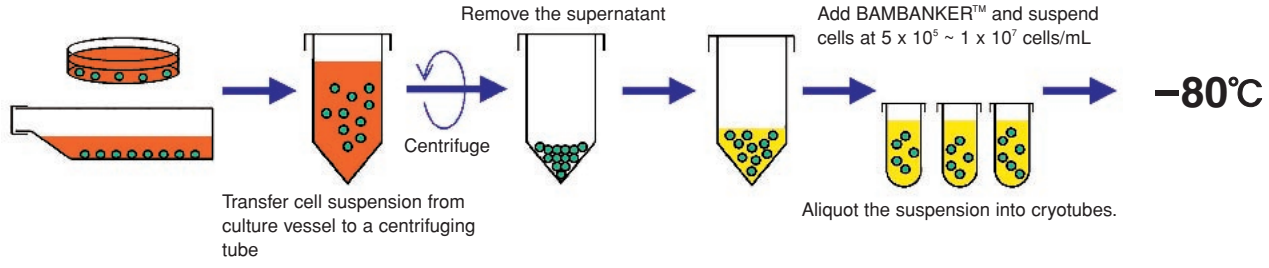
BAMBANKER™ is a serum-free medium for long-term freezing at -80℃ and preservation of valuable culture cells such as tumor cells and normal cells.



[Features]

1. **Ready-to-use medium for preservation of cells**
2. **Usable without diluting**
3. **No program freezer is required**
4. **Rapid & long-term freezing for preservation in a deep freezer (-80℃)**
5. **Serum-free**

[Operating Procedure]



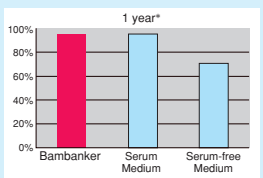
[Comparison of Bambanker™ with other relevant products]

−Cryopreservation Test−

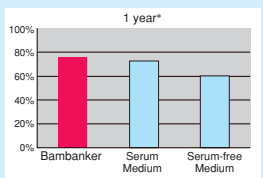
<Result> **∗ : Storage period of Cell Line at -80℃**

Media tested
1. **Bambanker™** (LYMPHOTEC Inc.)
2. Medium with serum (Company A)
3. Serum-free Medium (Company A)

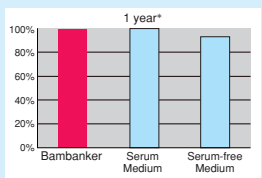
P3U1 (mouse myeloma cell line)
Cell number/vial 2.0 × 10^6



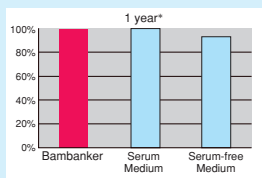
K562 (human leukemia cell line)
Cell number/vial 3.0 × 10^6



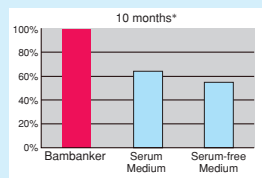
OKT4 (mouse hybridoma)
Cell number/vial 1.3 × 10^6



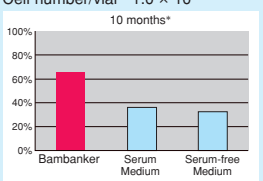
Daudi (human B cell line)
Cell number/vial 9.2 × 10^5



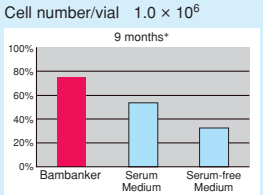
human gastric epithelial cells
Cell number/vial 1.0 × 10^6



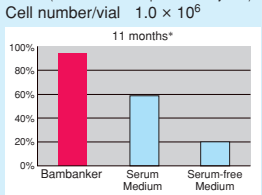
human γδT cells
Cell number/vial 1.0 × 10^6



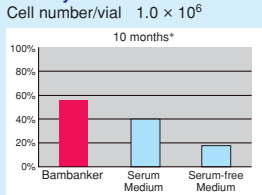
human B cell line
Cell number/vial 1.0 × 10^6



PC12 (rat-derived adrenal pheochromocytoma)
Cell number/vial 1.0 × 10^6



monkey B cell line
Cell number/vial 1.0 × 10^6



Wako Cat. No.	Description	Package Size	Storage
302-14681	BAMBANKER™	120 mL	Keep at 2 ~ 10℃

BAMBANKER™ is manufactured by **LYMPHOTEC Inc.** (Tokyo, Japan)

- Listed products are intended for laboratory research use only, but not to be used for drug, food or human use.
- Please visit our online catalog to search for other products from Wako ; **http://search.wako-chem.com**
- This brochure may contain products that cannot be exported to your country due to regulations.
- Bulk quote requests for some products are welcomed. Please contact us.

04X15IBK

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Fax: 81-6-6201-5964
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http://search.wako-chem.com

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http://www.wakousa. com
Head Office:
1600 Bellwood Road, Richmond, VA 23237
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