MBL

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PURIFICATION that Maintains Protein Activity

c-Myc tagged Protein
MILD PURIFICATION GEL

(MoAb. clone 1G4)

CODE No. 3306, 3307

Purification with neutral pH (to maintain Activity and Conformation)

High Quality and Efficiency

Rapid, High-Yield Purification

MBL MEDICAL & BIOLOGICAL LABORATORIES CO., LTD.
URL http://ruo.mbl.co.jp
e-mail support@mbl.co.jp, TEL 052-238-1904

Product Description

The ability to isolate and study a purified protein lies at the heart of modern biochemistry. Researchers in many fields require highly purified, active proteins for studies involving signaling pathways, enzymology, receptor binding, DNA binding, post-transcriptional modifications, and much more.

The method of purification is one of the important keys for maintaining protein structure and function. The c-Myc tagged Protein MILD PURIFICATION GEL is designed for the isolation of c-Myc tagged protein from cell lysates and culture supernatant. Severe conditions such as acidic or alkaline elution can denature protein structure while maintaining a neutral pH can preserve protein activity and conformation. MBL has developed the Anti-c-Myc tag Gel to quickly and efficiently purify c-Myc tagged proteins at neutral pH to maintain protein activity and native conformation. The c-Myc tag peptide competitively elutes the c-Myc tagged protein from Anti-c-Myc tag Gel while minimally interfering with protein function.

The affinity of the anti-c-Myc tag antibody is also very important. Very high affinity antibody cannot be dissociated from the c-Myc tagged protein, and low affinity antibody is not sufficient for binding. MBL has optimized the affinity of the anti-c-Myc tag antibody to permit high yields and efficient purification of functional c-Myc-tagged proteins for biochemical studies.

Anti-c-Myc tag monoclonal antibody (clone 1G4) used by this gel shows the best affinity to refine the c-Myc tag fusion protein under a mild condition.

Components

	Quantity	
	CODE No. 3306	CODE No. 3307
Anti-c-Myc tag Gel	1 mL × 1	1 mL × 5
50 % slurry: 1 mL Gel in 2 mL total volume in PBS with 0.1% ProClin 150 as preservative		
Elution Peptide	1 mg × 1	1 mg × 5
Lyophilized form: c-Myc tag peptide (EQKLISEEDL), reconstitute the Elution Peptide with 1 mL of distilled water. 1 mg in 1 mL PBS after reconstitution		

Product Capacity

The purification capacity of the Anti-c-Myc tag Gel varies depending upon the c-Myc tagged protein. For example, 1 mL of Anti-c-Myc Gel bound 1.0 mg of a c-Myc tagged protein (27 kDa) and eluted 0.64 mg of purified protein in our hands.

Storage

Store for up to 1 year from date of receipt at 2-8°C. Do not freeze.

Material Preparation

Prepare the following reagents before affinity purification.

Equilibration buffer	: PBS
Elution buffer	: 0.1-0.5 mg/mL c-myc tag peptide (EQKLISEEDL) in Equilibration buffer. Fully reconstitute the Elution peptide with 1 mL of distilled water, and mix it with 1-9 mL of Equilibration buffer in another tube. Store the reconstituted Elution peptide in aliquots at -20°C.
Regeneration buffer	: 0.17 M Glycine-HCl, (pH 2.3).
Column storage buffer	: PBS/Preservative (e.g. 0.1% ProClin 150 and 0.09% NaN_3).

Protocols

Notes:
1. Cellular debris and particulate matter must be removed prior to purification. The protein extract should be centrifuged (10,000-20,000 × g for 15 min) and filtered with a 0.45 μm filter to remove any remaining cells and particulates.
2. Highly viscous samples containing chromosomal DNA or RNA should be sonicated or treated with nuclease to reduce viscosity.

A. Column preparation

1. Place the empty chromatography column on rack or stand.
2. Rinse the column with Equilibration buffer.
3. Resuspend Anti-c-Myc tag Gel by tapping and inverting the vial several times immediately before dispensing. Don't vortex.
4. Transfer the desired volume to the column. Drain column storage buffer.
   Do not allow the column to dry out.
5. Wash the resin with 10 bed-volumes of Regeneration buffer.
6. Immediately wash the resin with 10 bed-volumes of Equilibration buffer.

B. Loading column

1. Load the lysate on the top of the column under gravity flow.
   Note: The binding efficiency depends on the fusion protein and on the loading flow rate.
   The binding efficiency may be increased by multiple passes over the column.
2. Collect the flow-through into clean collection tubes
3. Wash the column with PBS until the OD280 is <0.01

C. Elution

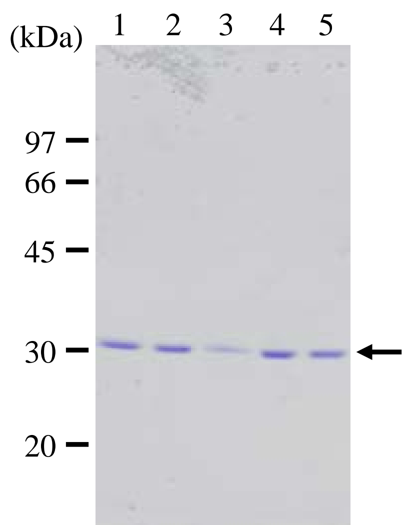
1. Elute the bound protein from the column with 5 bed-volumes of Elution buffer.
2. Collect the elution fraction into clean collection tubes.

D. Regeneration and storage

1. Wash the column with 10 bed-volumes of Regeneration buffer.
2. Immediately wash the column with 10 bed-volumes of Column storage buffer.
3. Store at +2 to +8°C in 2 bed-volumes of Column storage buffer.
   Note: Poured columns containing the anti-c-Myc tag Gel may be used at least 10 times, depending on the usage conditions.

Example of Purification Results
using c-Myc tagged Protein MILD PURIFICATION GEL

Elution under various conditions (using spin column.)

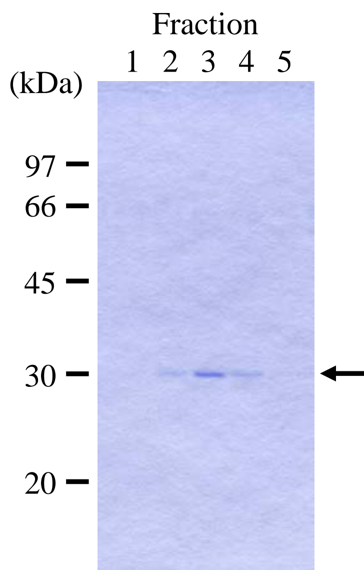


Lane 1: 0.1 mg/mL peptide
Lane 2: 1.0 mg/mL peptide
Lane 3: pH 4.0
Lane 4: pH 3.0
Lane 5: pH 2.7

The c-Myc tag fusion protein was purified under various conditions. The amount of eluted protein was nearly identical for all tested conditions excluding pH 4.0.

SDS-PAGE (Coomassie Brilliant Blue Staining)

Purification using open column

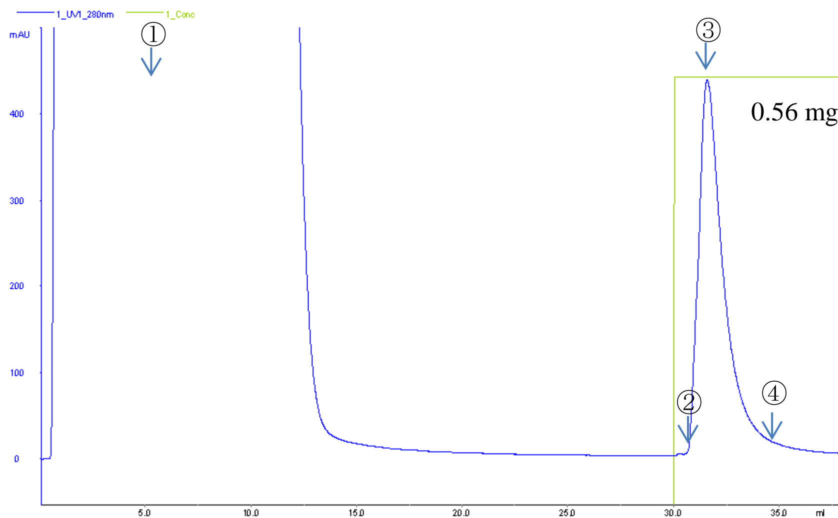


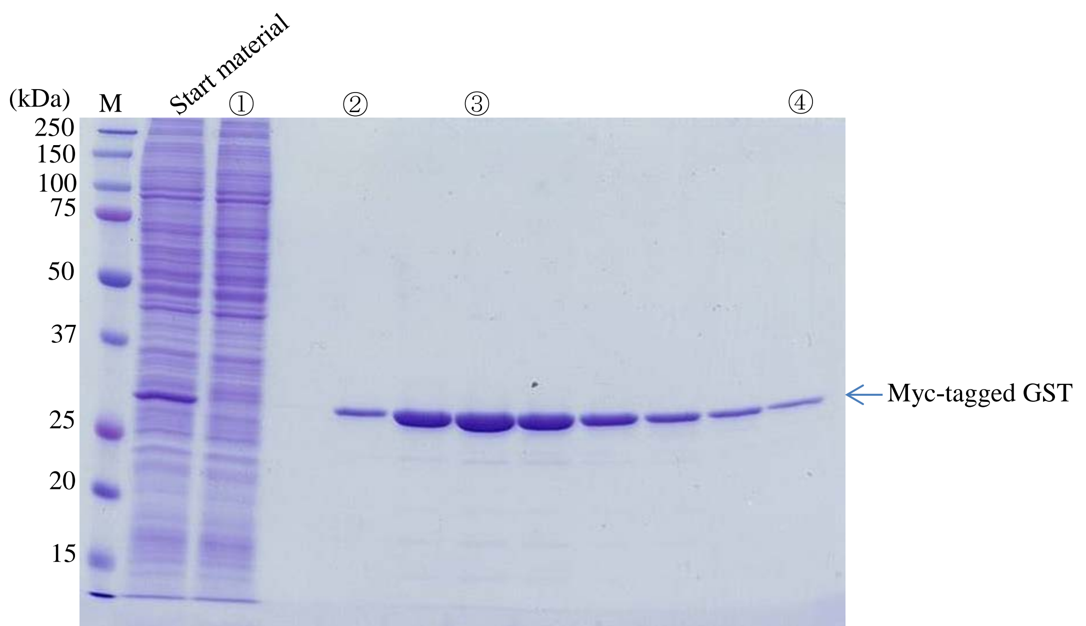
The c-Myc tagged protein was loaded onto the open column (bed vol. ~0.5 mL). Elution was carried out with 0.1 mg/mL peptide. Each fraction was 0.5 mL.

SDS-PAGE (Coomassie Brilliant Blue Staining)

Purification using HPLC system

N-terminus c-Myc-tagged GST





Column bed volume : 1 mL

Sample : c-Myc-tagged GST/293T (5×10^7 cells)

Cell lysis buffer : 10 mM Tris-HCl, 150 mM NaCl, 1% NP-40 (pH 7.5)

Wash buffer : 0.05% Tween/PBS (pH 7.2)

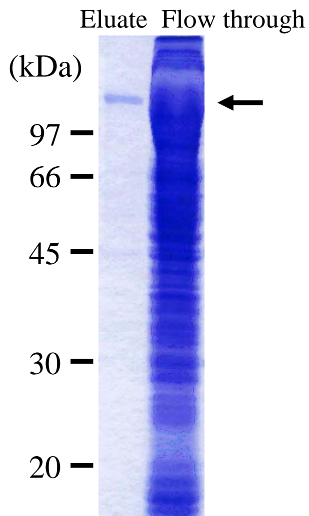
Elution buffer : 0.5 mg/ml c-Myc peptide in PBS

Flow rate : 0.75 mL/min

Chromatography system : ÄKTA explorer 10S (GE Healthcare)

Purification of c-Myc tagged protein from mammalian cells

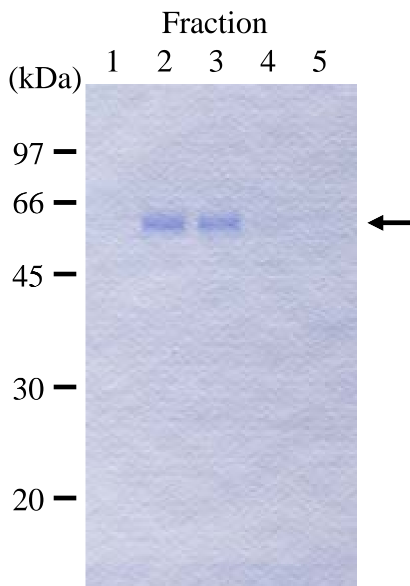
c-myc tagged SIRT1/Sir2 Deacetylase (N-terminal tagged, using spin column)



Human embryonic kidney cells (293T) were transfected and cultured for 70 hours. Cells were then lysed in the Lysis buffer (500 μL/100 mm dish) and purified using a spin column. Elution was carried out with 0.1 mg/mL c-Myc tag peptide.

SDS-PAGE (Coomassie Brilliant Blue Staining)

c-Myc tagged GFRA3 (C-terminal tagged, using open column)



Human embryonic kidney cells (293T) were transfected and cultured for 70 hours. Culture supernatant was loaded onto the column (bed vol. 0.5 mL). Elution was carried out with 2.5 mL of 0.1 mg/mL c-Myc tag peptide. Each fraction was 0.5 mL.

SDS-PAGE (Coomassie Brilliant Blue Staining)

Additional Information

Several reagents were examined whether or not they were suitable for use with the c-Myc tagged Protein MILD PURIFICATION GEL. The results are listed below.

Chaotropic agents

	Reagent	Concentration	Result
	Urea	1 M	Yes
	Guanidine-HCl	1 M	No

Reducing agents

	Reagent	Concentration	Result
	DTT	10 mM	Yes
	2-Mercaptoethanol	10 mM	Yes

Surfactants

	Reagent	Concentration	Result
Nonionic	Tween-20	5%	Yes
	TritonX-100	5%	Yes
	NP40	5%	Yes
	Digitonin	1%	Yes
	n-Octyl-^{2} -D-gulcoside	1%	Yes
Zwitterionic	CHAPS	1%	Yes
	CHAPSO	1%	Yes
Anionic	SDS	0.05%	No
	Sodium Deoxycholate	0.1%	Yes
	Sodium Deoxycholate	0.5%	No

Others

	Reagent	Concentration	Result
	NaCl	1 M	Yes
	Glycerol	10%	Yes
	EDTA	10 mM	Yes

The “Yes” indicates the reagents can be used in the Lysis buffer for this Gel up to the indicated concentration. The “No” indicates the reagents cannot be used in the Lysis buffer for this Gel at the indicated concentration.

RELATED PRODUCTS:

3305	c-Myc-tagged Protein Mild Purification Kit ver.2	20 purifications
3305A	c-Myc-tagged Protein Mild Purification Kit ver.2	2 purifications
3340	c-Myc-tagged Protein Magnetic Purification Kit	1 kit
3340A	c-Myc-tagged Protein Magnetic Purification Kit (Trial Kit)	1 kit
3306	c-Myc-tagged Protein Mild Purification Gel with Elution Peptide	Gel: 1 mL x 1, Peptide: 1 mg x 1
3307	c-Myc-tagged Protein Mild Purification Gel with Elution Peptide	Gel: 1 mL x 5, Peptide: 1 mg x 5
3306K	c-Myc-tagged Protein PURIFICATION CARTRIDGE	1 mL × 1
3300-205	c-Myc-tag peptide	1 mg x 5
3310	His-tagged Protein Purification Kit	20 purifications
3310A	His-tagged Protein Purification Kit (Trial Kit)	2 purifications
3311	His-tagged Protein Purification Gel with Elution Peptide	Gel: 1 mL x 1, Peptide: 2 mg x 5
3312	His-tagged Protein Purification Gel with Elution Peptide	Gel: 1 mL x 5, Peptide: 2 mg x 25
3310-205	His-tag peptide	2 mg x 5
3317	V5-tagged Protein Purification Kit Ver.2	20 purifications
3317A	V5-tagged Protein Purification Kit Ver.2 (Trial Kit)	2 purifications
3341	V5-tagged Protein Magnetic Purification Kit	1 kit
3341A	V5-tagged Protein Magnetic Purification Kit (Trial Kit)	1 kit
3318	V5-tagged Protein Purification Gel Ver.2	Gel: 1 mL x 1
3315-205	V5-tag peptide	2 mg x 5
3320	HA-tagged Protein Purification Kit	20 purifications
3320A	HA-tagged Protein Purification Kit (Trial Kit)	2 purifications
3342	HA-tagged Protein Magnetic Purification Kit	1 kit
3342A	HA-tagged Protein Magnetic Purification Kit (Trial Kit)	1 kit
3321	HA-tagged Protein Purification Gel	1 mL
3320-205	HA-tag peptide	2 mg x 5
3325	DDDDK-tagged Protein Purification Kit	20 purifications
3325A	DDDDK-tagged Protein Purification Kit (Trial Kit)	2 purifications
3343	DDDDK-tagged Protein Magnetic Purification Kit	1 kit
3343A	DDDDK-tagged Protein Magnetic Purification Kit (Trial Kit)	1 kit
3326	DDDDK-tagged Protein Purification Gel with Elution Peptide	Gel: 1 mL x 1, Peptide: 1 mg x 5
3327	DDDDK-tagged Protein Purification Gel with Elution Peptide	Gel: 1 mL x 5, Peptide: 1 mg x 25
3328	DDDDK-tagged Protein Purification Gel	5 mL x 1
3329	DDDDK-tagged Protein Purification Gel	5 mL x 5
3326K	DDDDK-tagged Protein PURIFICATION CARTRIDGE	1 mL × 1
3325-205	DDDDK-tag peptide	1 mg x 5

はじめに

さまざまな研究分野で､活性のあるタンパク質､構造を保ったタンパク質を精製することは大変重要です。活性や構造を保ったままでタンパク質を精製するためには、酸、アルカリなどの過酷な条件下ではなく、マイルドな中性条件下で精製できることが理想的です。このゲルは、哺乳動物細胞などで発現させた c-Myc tag 融合タンパク質をマイルドな中性条件下で精製可能にしたゲルです。

MBL では、このゲルのために、最適な親和性を持った抗体を開発しました。抗体と抗原の親和性が弱いと十分な結合が起こらず、強すぎると抗体と抗原の解離をマイルドな中性条件下では行うことができません。本ゲルで使用している抗 c-Myc tag モノクローナル抗体(clone 1G4)は c-Myc tag 融合タンパク質をマイルドな中性条件下で精製するために最適な親和性を示します。

構成

	Quantity	
	CODE No. 3306	CODE No. 3307
Anti-c-Myc tag Gel	1 mL × 1 本	1 mL × 5 本
Elution Peptide	1 mg × 1 本	1 mg × 5 本

Anti-c-Myc tag Gel:
50 % スラリー: 保存剤として 0.1%の ProClin 150 を含有する PBS に 1 mL のビーズが入り 2 mL となっています。

Elution Peptide:
凍結乾燥品: c-Myc tag peptide (EQKLISEEDL)
1 mL の脱イオン水を加えて溶解してください。
溶解後に 1 mg/mL の PBS 溶液となります。

精製のキャパシティー

精製のキャパシティーは c-Myc tag 融合タンパク質の種類によって異なります。
27 kDa の c-Myc tag 融合タンパク質で測定した例では、Anti-c-Myc tag Gel 1 mL あたり 1.0 mg の c-Myc tag 融合タンパク質と結合し、0.6 mg の c-Myc tag 融合タンパク質を回収することができました。

保存

製品有効期限は、出荷後 1 年間です。2-8°C で保存して下さい。凍結はお避け下さい。

試薬の準備

1. 平衡化バッファー: PBS
2. 溶出バッファー : 0.1-0.5 mg/mL c-Myc tag peptide (EQKLISEEDL) in PBS

   Elution Peptide に 1 mL の脱イオン水を加えて溶解した後、平衡化バッファーで 2～10 倍希釈してください。
   *ペプチド溶液を保存する場合は適切な量に分注して、-20°C に保存して下さい。
   凍結融解の繰り返しは避けて下さい。
3. 再生バッファー : 0.17 M Glycine-HCl, pH 2.3
4. 保存バッファー : PBS/Preservative (e.g. 0.1% ProClin 150 and 0.09% NaN_3)

プロトコル

サンプル中に微粒子が含まれている場合には精製前に取り除く必要があります。遠心処理（10,000 - 20,000 × g）した後、上清を 0.45 µm のフィルターに通して微粒子を除去してください。ゲノム DNA や RNA 等を含むサンプルで、粘性が高い場合には超音波処理または適当な試薬（ヌクレアーゼなど）で処理をして粘性を下げてください。

A. カラム準備

1. 空のカラムを鉛直に立てます。
2. カラムを適当量の平衡化バッファーで洗浄します。
3. Anti-c-Myc tag Gel の容器を指ではじき転倒混和して均一なスラリーにしてください。ボルテックスは使わないでください。
4. 必要量の Anti-c-Myc tag Gel をカラムに入れ保存液を排出します。
5. カラムボリュームの 10 倍量の再生バッファーでカラムを洗浄します。
6. 直ちにカラムボリュームの 10 倍量の平衡化バッファーを流します。
   *ゲルベッドを乾燥させないでください。

B. c-Myc tag 融合タンパク質のゲルへの吸着

1. 自然落下流速によりサンプルをカラムにローディングします。
   （注意：c-Myc tag 融合タンパク質の種類、流速、温度などの条件によって Anti-c-Myc tag Gel への結合効率が変わることがあります。結合効率が低い場合には①カラムにサンプルを複数回通す、②サンプルと Anti-c-Myc tag Gel を適当な容器に入れて混合し穏やかに攪拌しながらインキュベートすることにより改善することがあります。）
2. 素通り画分をチューブに回収します。
3. カラムを平衡化バッファーで洗浄し、OD280 が 0.01 以下になるまで洗浄して下さい。

C. c-Myc tag 融合タンパク質の溶出

1. ベッドボリュームの 5 倍量の溶出バッファーで溶出させます。
2. 溶出液を適当なチューブに回収します。
   （注意：c-Myc tag 融合タンパク質、流速、温度などの条件によりにより溶出効率が影響を受けることがあります。）

D. 再生及び保存

1. カラムをベッドボリュームの 10 倍量の再生バッファーで洗浄します。
2. 直ちにベッドボリュームの 10 倍量以上の保存バッファーで洗浄し、排出液の pH が中性に戻っていることを確認します。
3. 保存バッファーを加えて密閉し 2～8°C で保存します。
   *使用条件により異なりますが 10 回程度は使用できます。

関連製品

3305	c-Myc-tagged Protein Mild Purification Kit ver.2	20 purifications
3305A	c-Myc-tagged Protein Mild Purification Kit ver.2	2 purifications
3340	c-Myc-tagged Protein Magnetic Purification Kit	1 kit
3340A	c-Myc-tagged Protein Magnetic Purification Kit (Trial Kit)	1 kit
3306	c-Myc-tagged Protein Mild Purification Gel with Elution Peptide	Gel: 1 mL x 1, Peptide: 1 mg x 1
3307	c-Myc-tagged Protein Mild Purification Gel with Elution Peptide	Gel: 1 mL x 5, Peptide: 1 mg x 5
3306K	c-Myc-tagged Protein PURIFICATION CARTRIDGE	1 mL × 1
3300-205	c-Myc-tag peptide	1 mg x 5
3310	His-tagged Protein Purification Kit	20 purifications
3310A	His-tagged Protein Purification Kit (Trial Kit)	2 purifications
3311	His-tagged Protein Purification Gel with Elution Peptide	Gel: 1 mL x 1, Peptide: 2 mg x 5
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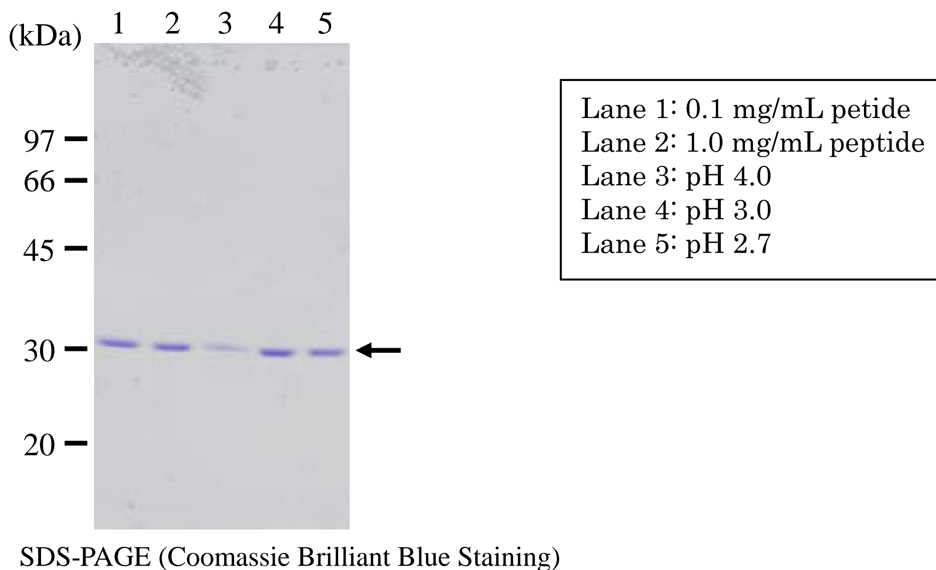
3328	DDDDK-tagged Protein Purification Gel	5 mL x 1
3329	DDDDK-tagged Protein Purification Gel	5 mL x 5
3326K	DDDDK-tagged Protein PURIFICATION CARTRIDGE	1 mL × 1
3325-205	DDDDK-tag peptide	1 mg x 5

c-Myc tagged Protein MILD PURIFICATION GEL を用いた精製例

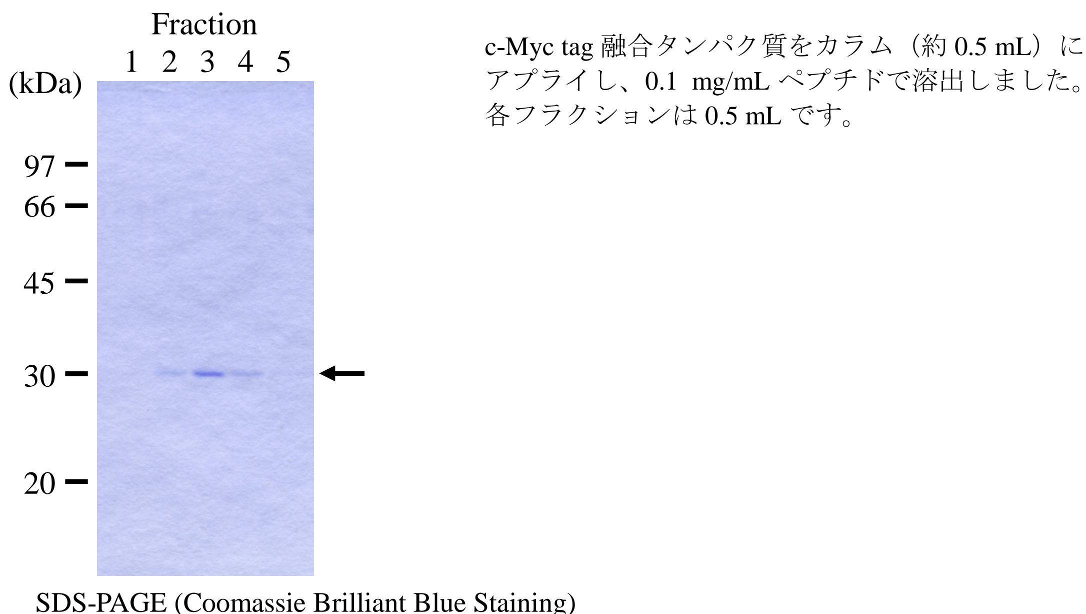
さまざまな条件下での溶出例（スピンカラム使用）



SDS-PAGE (Coomassie Brilliant Blue Staining)

c-Myc tag 融合タンパク質をさまざまな溶出条件下で精製しました。溶出されたタンパク質量は pH 4.0 での溶出を除きほとんど同じ結果となりました。

オープンカラムを用いた精製

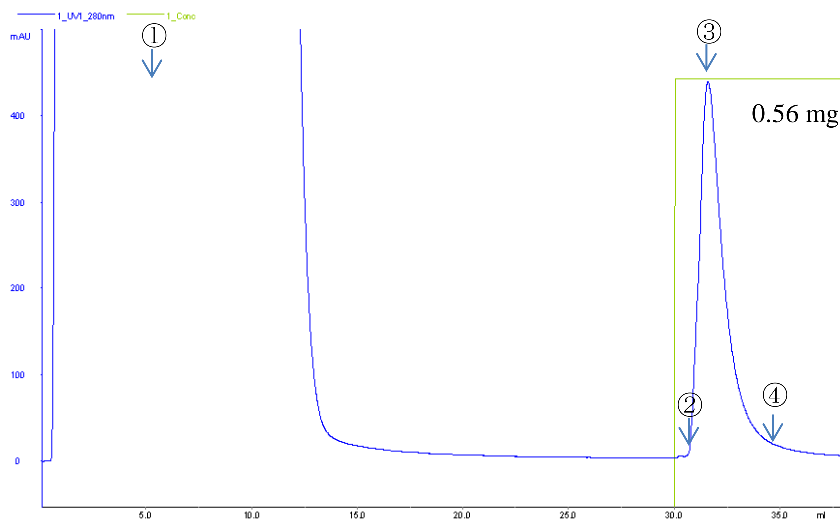


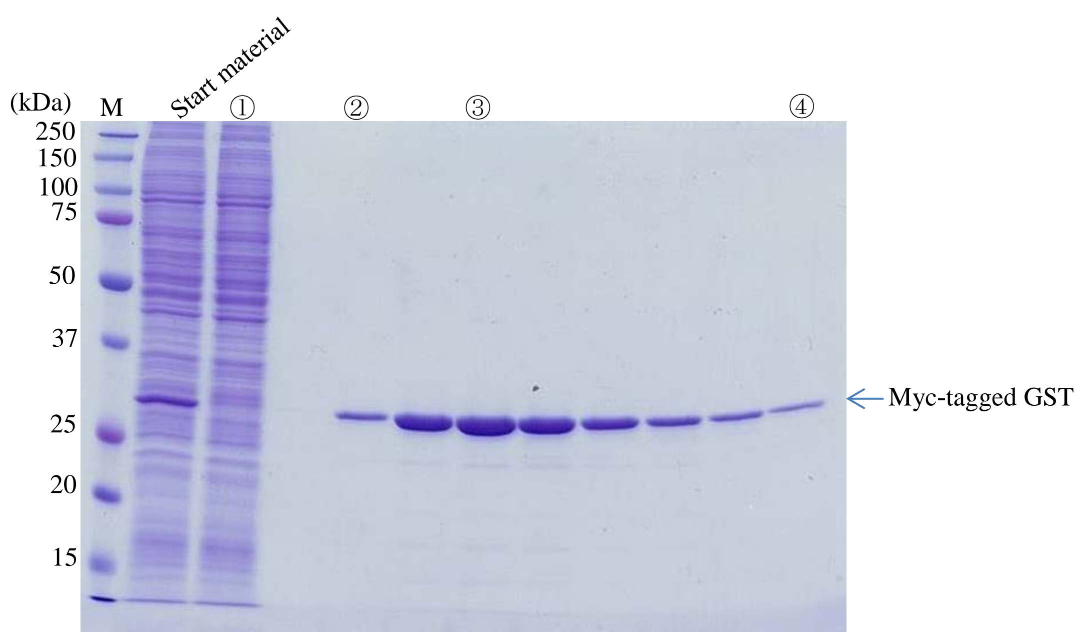
c-Myc tag 融合タンパク質をカラム（約 0.5 mL）にアプライし、0.1 mg/mL ペプチドで溶出しました。各フラクションは 0.5 mL です。

SDS-PAGE (Coomassie Brilliant Blue Staining)

HPLC システムを用いた精製

N-terminus c-Myc-tagged GST

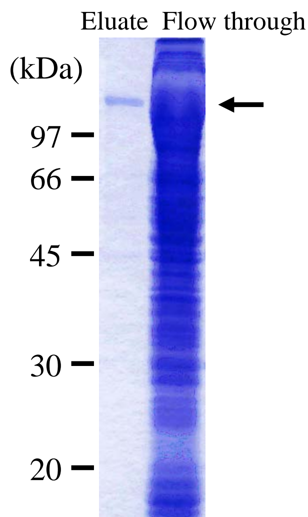




Column bed volume	: 1 mL
Sample	: c-Myc-tagged GST/293T ($5x10^7$cells)
Cell lysis buffer	: 10 mM Tris-HCl, 150 mM NaCl, 1% NP-40 (pH 7.5)
Wash buffer	: 0.05% Tween/PBS (pH 7.2)
Elution buffer	: 0.5 mg/ml c-Myc peptide in PBS
Flow rate	: 0.75 mL/min
Chromatography system	: ÄKTAexplorer 10S (GE Healthcare)

哺乳動物細胞からの **c-Myc tag** 融合タンパク質の精製

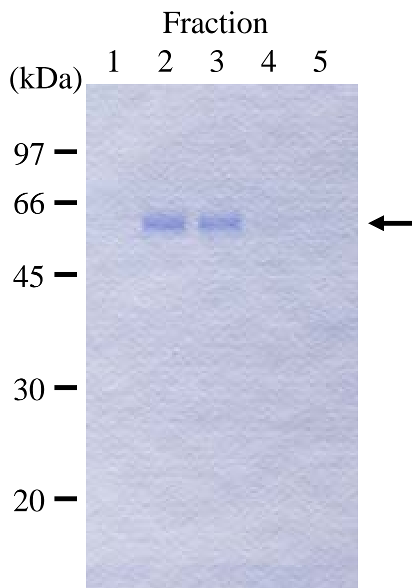
c-Myc tagged SIRT1/Sir2 Deacetylase (N-terminal tagged, using spin column)



ヒト胎児腎由来細胞株（293T）に遺伝子をトランスフェクションし 70 時間培養しました。細胞を細胞溶解バッファーに溶解させスピンカラムを用いて精製しました。
溶出は 0.1 mg/mL ペプチド溶液で行いました。

SDS-PAGE (Coomassie Brilliant Blue Staining)

c-Myc tagged GFRA3 (C-terminal tagged, using open column)



ヒト胎児腎由来細胞株（293T）に遺伝子をトランスフェクションし 70 時間培養しました。培養上清をオープンカラム(ベッド体積 0.5 mL)にアプライし 2.5 mL の 0.1 mg/mL ペプチド溶液で溶出しました。各フラクションは 0.5 mL です。

SDS-PAGE (Coomassie Brilliant Blue Staining)

試薬の使用可否

下記の試薬を細胞溶解バッファーの成分に加えた場合、本ゲルで使えるか調べました。

Chaotropic agents

	Urea	1 M	Yes
	Guanidine-HCl	1 M	No

Reducing agents

	DTT	10 mM	Yes
	2-Mercaptoethanol	10 mM	Yes

Surfactants

Nonionic	Tween-20	5%	Yes
	TritonX-100	5%	Yes
	NP40	5%	Yes
	Digitonin	1%	Yes
	n-Octyl-² -D-gulcoside	1%	Yes
Zwitterionic	CHAPS	1%	Yes
	CHAPSO	1%	Yes
Anionic	SDS	0.05%	No
	Sodium Deoxycholate	0.1%	Yes
	Sodium Deoxycholate	0.5%	No

Others

	NaCl	1 M	Yes
	Glycerol	10%	Yes
	EDTA	10 mM	Yes

Yes：表に示した濃度まで細胞溶解バッファーに加えて使用できます。

No：表に示した濃度で細胞溶解バッファーに加えると使用できません。

発売元

MBL 株式会社　医学生物学研究所

URL　http://ruo.mbl.co.jp

e-mail　support@mbl.co.jp

TEL　052-238-1904