Catalog No. 70-310

Anti-Nup98 antibody, rat monoclone (2H10)

BACKGROUND

Nucleoporin 98 (Nup98) is a component of nuclear pore complex (NPC), which is a large protein assembly embedded in the nuclear envelope. It is localized on both nuclear and cytoplasmic side of NPC. This protein contains glycine-leucine-phenylalanine-glycine (GLFG) amino acid repeats and plays a critical role in nuclear trafficking. Nup98 plays a specific role in the RNA export. Nup98 gene is fused to a variety of partner genes in human myeloid and T-cell malignancies via chromosomal translocation. This hybridoma has been established by Prof. T. Tachibana's Lab. at Osaka City Univ.

Applications:

1) Western blotting (Fig. 1), 2) Immunocytochemistry (Fig. 2), 3)ELISA, 4) Dot blotting

Properties: When injected into the cytoplasm, this antibody accumulates into the nuclear pores of HeLa cells and inhibits nuclear localization of endogenous Ran.

Immunogen: Recombinant GST-fused human Nup98 (amino acids 1-466)

Isotype: Rat IgG2c (κ)

Reactivity: Human, mouse, rat and S. pombe Nup98 protein. S. cerevisia nucleoporins, Nup116, Nup100, Nup145N, Nup57, Nup49. This antibody does not react with Tetrahymena. Other species have not been tested.

Form: Purified IgG (1 mg/ml) in PBS(-), 50% glycerol, filter-sterilized. Azide and carrier free

Size: 100ug

Storage: Shipped at 4℃ or -20℃, and upon arrival, spin-down and store at -20℃.

Data Link: UniProtKB/TrEMBL **Q9HDC8** (Q9HDC8_HUMAN)

References: This product has been described and used in reference 1 and 2.

1. Fukuhara T *et al* "Specific monoclonal antibody against the nuclear pore complex protein, nup98." *Hybridoma* **24**: 244-247 (2005) PMID: 16225424 **WB, IF**
2. Iwamoto M. et al. (2013) Monoclonal antibodies recognize gly-leu-phe-gly repeat of nucleoporin nup98 of tetrahymena, yeasts, and humans. Monoclon Antib Immunodiagn Immunother. 32: 81-90
PubMed ID: 23607342 **WB, IF**

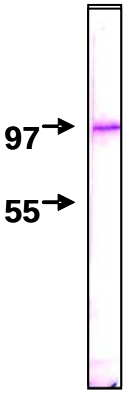

Fig.1 Detection of Nup98 protein by Western blotting using this antibody, 2H10.
The sample is HeLa nuclear membrane fraction.
The IgG was diluted 2,000 fold before use.

Anti-Nup98 antibody

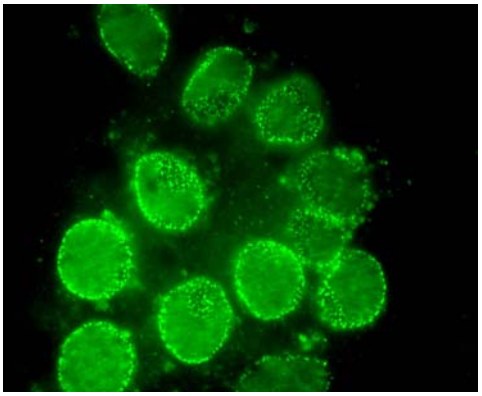

Fig. 2. Immunofluorescent staining of rat neuron with antibody 2H10.
The dots are Nup98.

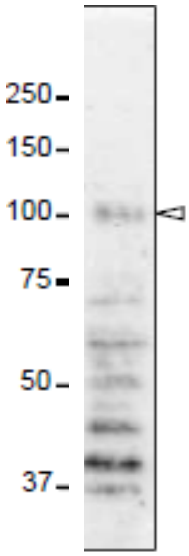

Fig. 3. Detection of Np98 protein in whole cell extract of HeLa cells with 2H10 antibody.

First antibody was used at 0.4 ug/ml and as second antibody, HRP-conjugated ant-rat IgG was used at 0.4 ug/ml. The arrow head indicates the position of Nup98 protein. The numbers on the left shows the positions of size marker proteins in kDa.

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Anti-Nup98 antibody

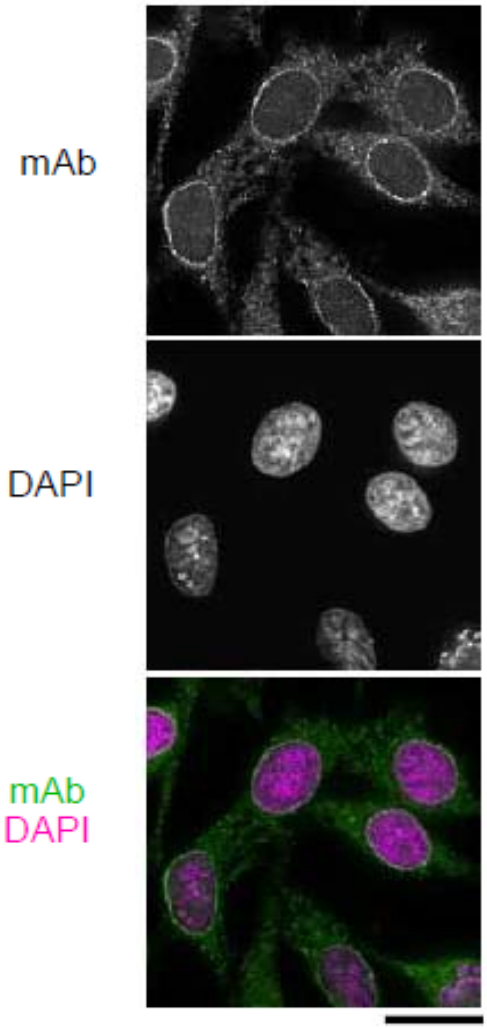

Fig.4 Immunofluorescene staining of Nup98 in HeLa cells with 2H10 antibodies. Black-and-white images were obtained with mAb and DAPI. Color images represent merged images of mAb (green) with DAPI (magenta).

HeLa cells were cultured in a glass-bottom dish for 2 days before fixation. The cells were fixed with cold methanol (-30°C) for 30 min. After washing with PBS, all fixed samples were blocked with 1% BSA for 2 hr at room temperature. Anti-Nup98 rat mAb 2H10 was diluted to 0.5 µg/ml in PBS. Blocked samples were treated with these primary antibody solutions overnight at 4°C. The secondary antibodies were 4 µg/ml of Alexa488-labelled anti-rat IgG. DNA was stained with 4',6-diamidino-2-phenylindole (DAPI). Samples were washed three times with PBS between treatments.

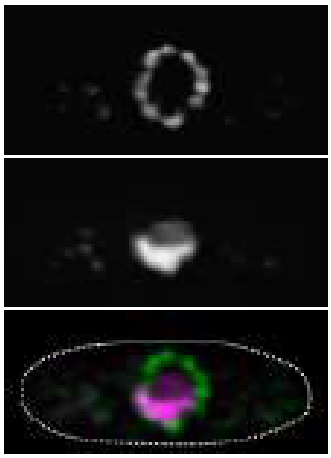

Fig. 5. Immunofluorescene staining of Nup98 in S. pombe cells with 2H10 antibody. Black-and-white images were obtained with mAb and DAPI. Color images represent merged images of mAb (green) with DAPI (magenta). Dotted lines represent the outlines of cells. *S. pombe* cells were fixed with 4% formaldehyde for 10 min, treated with 0.6 mg/ml Zymolyase 100T at 36°C for 70 min, and permeabilized with 1% Triton X-100 for 1 min. 10 µg/ml IgG solution of 2H10 were used. Antibody 2H10 stained the nuclear periphery of *S. pombe* in a punctate pattern.

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Anti-Nup98 antibody

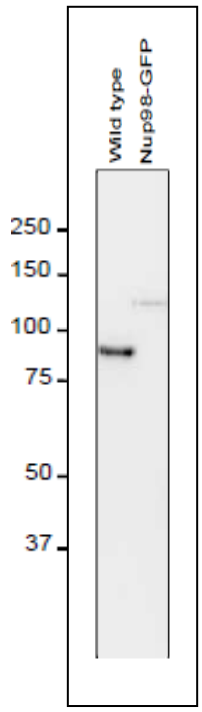

Fig. 6. Detection of Nup98 in S. pombe cell extracts by Western blotting with antibodies 2H10. 1 µg/ml IgG solution of 2H10 were used. Left and right lanes represent specimens from a wild type strain and an *S. pombe* strain in which Nup98 was chromosomally replaced with Nup98-GFP, respectively. S. pombe cells harvested were heated in distilled water at 95°C for 5 min, suspended in 100 mM phosphate buffer (pH 6.8), 4 M urea, 2.5% SDS, 0.05 mM EDTA, and disrupted by glass beads using a Multi Beads Shocker (Yasui Kikai, Osaka,). The sample was clarified by centrifugation, and the supernatant was used as the whole cell extract. First antibody was used at 0.4 ug/ml and as second antibody, HRP-conjugated ant-rat IgG was used at 0.4 ug/ml. Numbers on the left are positions of size marker

Proteins in kDa.

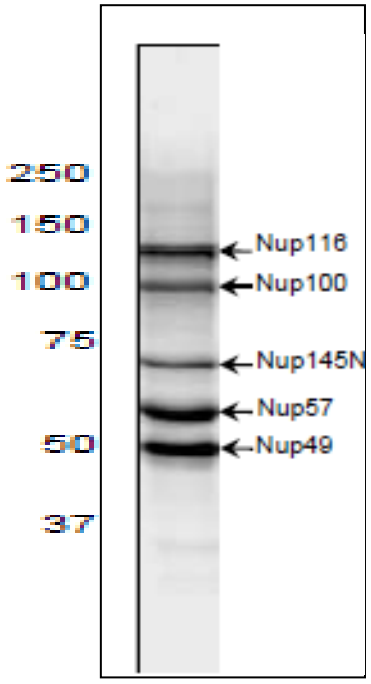

Fig. 7. Western blot analysis of *S. cereviciae* cell extract with 2H10 antibody. 2 µg/ml IgG solution of 2H10 were used. Arrows represent the positions of the indicated nucleoporins. Methods were as described for S. pombe.

Five protein bands identified by this antibody correspond to the sizes of 5 kinds of nucleoporins with multiple GFLG motifs in S. pombe.

Related Products:

70-345 anti-Nup98 antibody, mouse monoclonal 13C2

70-347 anti-Nup98 antibody mouse monoclonal 13C2+21A10.

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Catalog No. 70-310

抗 Nup98 抗体, ラットモノクローン(2H10)

Nucleoporin 98 (Nup98) は核膜に埋め込まれた大きな構造体である核膜孔(NPC)の構成成分の一つであり、核膜孔の両側に存在する。このタンパク質は Gly-Leu-Phe-Gly の繰り返し配列を持っていて、タンパク質の核移行及び RNA の核外への移行に必須である(1)。Nup98 遺伝子は白血病患者の染色体上で転移していて種々の遺伝子と融合している(2)。このハイブリドーマは大阪市立大学の立花太郎博士の研究室で作成された(3)。

用途:

1)ウエスターンブロッテイング　2)免疫染色　3)ELISA　4) ドットブロッテイング

性質: 本抗体を細胞質に注入すると核膜孔に蓄積し、内在性の Ran タンパク質の核局在を阻害する。

抗原: 組換え体 Nup98 タンパク質 (アミノ酸 1-466)

Isotype: Rat IgG2c (κ)

反応性: ヒト、マウス、ラット、分裂酵母の Nup98 タンパク質及び出芽酵母の Nup98 関連ヌクレオポリン, Nup116, Nup100, Nup145N, Nup57, Nup49。テトラヒメナとは反応しない。他の生物種では調べていない。

性状: 精製 IgG (1 mg/ml) in PBS(-), 50% glycerol, filter-sterilized. Azide- and carrier-free

容量：100ug

保存: 輸 送：4℃ または -20℃. 到着後、遠心して抗体液を落としてから-20℃で保存。

データリンク: UniProtKB/TrEMBL **Q9HDC8** (Q9HDC8_HUMAN)

文献: 本抗体は文献 1, 2 に記載され, 使用されている。

1. Fukuhara T *et al* "Specific monoclonal antibody against the nuclear pore complex protein, nup98." *Hybridoma* **24**: 244-247 (2005) PMID: 16225424 **WB,IF**

2. Iwamoto M. et al. (2013) Monoclonal antibodies recognize gly-leu-phe-gly repeat of nucleoporin nup98 of tetrahymena, yeasts, and humans. Monoclon Antib Immunodiagn Immunother. 32: 81-90
PubMed ID: 23607342 **WB, IF**

抗 POGZ 抗体

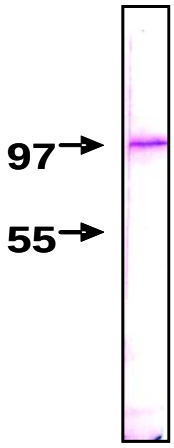

図 1　Nup98 タンパク質の 2H10 抗体を用いたウエスタンブロッティングによる検出。
サンプルは HeLa 細胞の核膜画分。
抗体は 2,000 倍希釈で用いた。

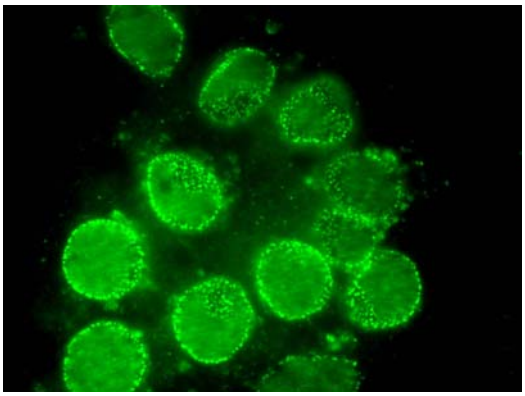

図 2 マウス神経細胞の 2H10 抗体を用いた免疫抗体染色。
Nup98 タンパク質は粒子状にみえる。

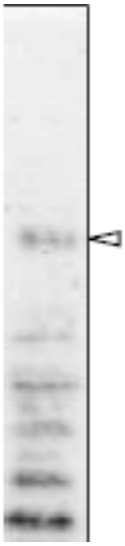

図３．HeLa 細胞全抽出液中の Nup98 タンパク質の抗 Nup98 抗体(2H10) を用いたウエスターンブロットによる検出。

一次抗体は 2,500 倍希釈で使用した。二次抗体は HRP コンジュゲートした抗ラット IgG 抗体を 0.4 ug/ml の濃度で用いた。

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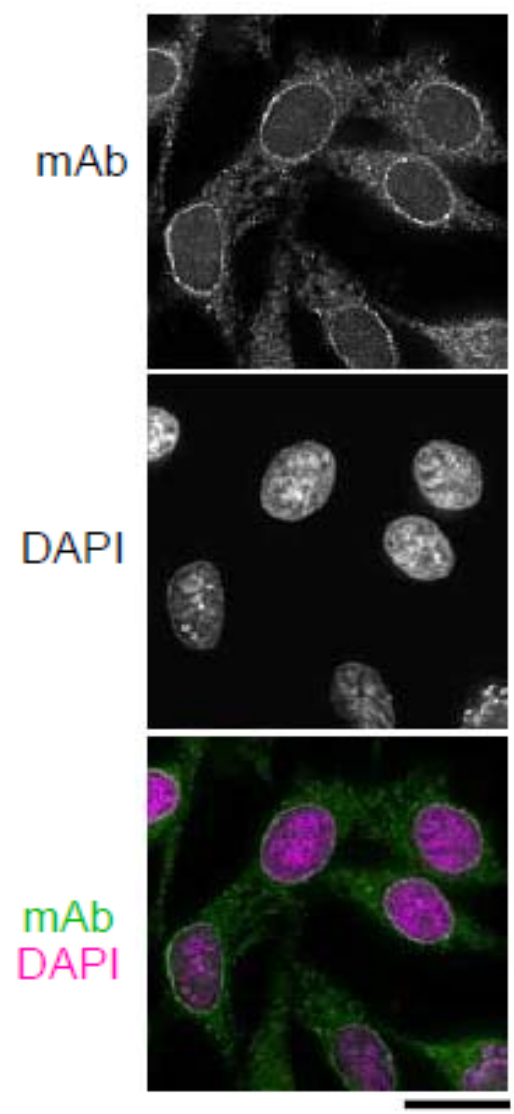

図４．抗 Nup98 抗体(2H10) を用いた間接免疫蛍光染色法による HeLa 細胞中の Nup98 タンパク質の検出。

細胞はメタノールで固定し、一次抗体は 0.5 ug/ml で使用し、二次抗体は Alexa488 ラベルした抗ラット IgG を 0.5 ug/ml の濃度で使用した。上段(mAb) は抗体による免疫蛍光染色、DNA は DAPI で染色し、下段のカラー像は抗体染色（緑色）と DAPI 染色（マジェンダ）を重ねた結果である。

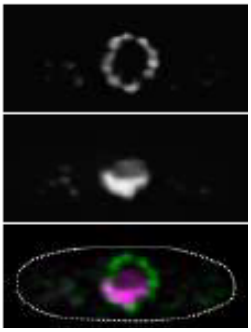

図５．抗 Nup98 抗体(2H10) を用いた間接免疫蛍光染色法による分裂酵母細胞中の Nup98 タンパク質の検出。

細胞はメタノールで固定し、一次抗体は 0.5 ug/ml で使用し、二次抗体は Alexa488 ラベルした抗ラット IgG を 0.5 ug/ml の濃度で使用した。上段(mAb) は抗体による免疫蛍光染色、DNA は DAPI で染色し、下段のカラー像は抗体染色（緑色）と DAPI 染色（マジェンダ）を重ねた結果である。点線で囲ってあるのは細胞の外周である。酵母細胞は 4% formaldehyde で固定し、0.6 mg/ml の Zymolyase 100T で処理し、1% Triton X-100 で透過処理した。一次抗体は 10 ug/ml の濃度を用いた。

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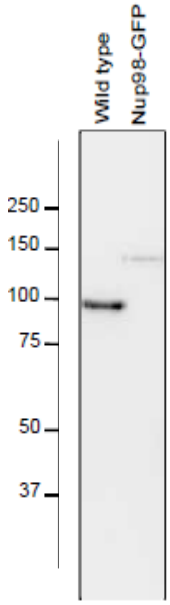

図６. 分裂酵母細胞粗抽出液中の Nup98 タンパク質の抗 Nup98 抗体(2H10) を用いたウエスターンブロットによる検出。

一次抗体は 1 ug/ml で使用した。二次抗体は HRP コンジュゲートした抗ラット IgG 抗体を 0.4 ug/ml の濃度で用いた。左は内在性の Nup98, 右は染色体上の Nup98 遺伝子を Nup98-GFP 遺伝子で置き換えた酵母からのサンプルである。

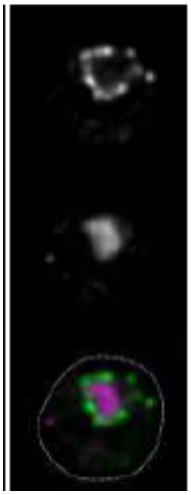

図７. 抗 Nup98 抗体(2H10) を用いた間接免疫蛍光染色法による出芽酵母細胞中の Nup98 タンパク質の検出。

細胞はメタノールで固定し、一次抗体は 10 ug/ml で使用し、二次抗体は Alexa488 ラベルした抗ラット IgG を 0.5 ug/ml の濃度で使用した。上段(mAb) は抗体による免疫蛍光染色、DNA は DAPI で染色し(中断)、下段のカラー像は抗体染色（緑色）と DAPI 染色（マジェンダ）を重ねた結果である。点線で囲ってあるのは細胞の外周である。酵母細胞は 4% formaldehyde で固定し、0.6 mg/ml の Zymolyase 100T で処理し、1% Triton X-100 で透過処理した。一次抗体は 10 ug/ml の濃度を用いた。

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