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Nucleostemin Recombinant Polyclonal Antibody (3HCLC)

Catalog Number 710188

Product data sheet

Details	
Size	100 µg
Host/Isotope	Rabbit / IgG
Class	Recombinant Polyclonal
Type	Antibody
Clone	3HCLC
Immunogen	Recombinant protein corresponding to amino acids 1-180 of human nucleostemin
Conjugate	Unconjugated
Form	Liquid
Concentration	0.5 mg/mL
Purification	Protein A
Storage buffer	PBS
Contains	0.09% sodium azide
Storage Conditions	Store at 4°C short term. For long term storage, store at -20°C, avoiding freeze/thaw cycles.

Species Reactivity	
Species reactivity	Human

Tested Applications	Dilution *
Western Blot (WB)	0.5-1 µg/mL
Immunocytochemistry (ICC/IF)	2-4 µg/mL

* Suggested working dilutions are given as a guide only. It is recommended that the user titrate the product for use in their own experiment using appropriate negative and positive controls.

Product specific information

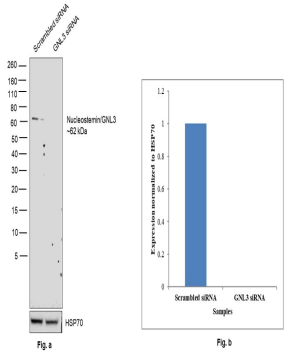
Recombinant rabbit polyclonal antibodies are unique offerings from Thermo Fisher Scientific. They are comprised of a selection of multiple different recombinant monoclonal antibodies, providing the best of both worlds - the sensitivity of polyclonal antibodies with the specificity of monoclonal antibodies - all delivered with the consistency only found in a recombinant antibody. While functionally the same as a polyclonal antibody - recognizing multiple epitope sites on the target and producing higher detection sensitivity for low abundance targets - a recombinant rabbit polyclonal antibody has a known mixture of light and heavy chains. The exact population can be produced in every lot, circumventing the biological variability typically associated with polyclonal antibody production.

Background/Target Information

Arylsulfatase F, also known as ARSF, is a 590 amino acid secretory protein that belongs to the sulfatase family of bone and cartilage matrix proteins. Arylsulfatase F uses calcium as a cofactor to catalyze reactions that are important in maintaining correct bone composition. The activity of Arylsulfatase F, unlike that of other family members, such as Arylsulfatase E, is not inhibited by warfarin. The gene encoding Arylsulfatase F maps to human chromosome X, which contains nearly 153 million base pairs and houses over 1,000 genes. In conjunction with chromosome Y, chromosome X is responsible for sex determination. There are a number of conditions related to an abnormal number and combination of sex chromosomes, some of which include Turner's syndrome, color blindness, hemophilia and Duchenne muscular dystrophy.

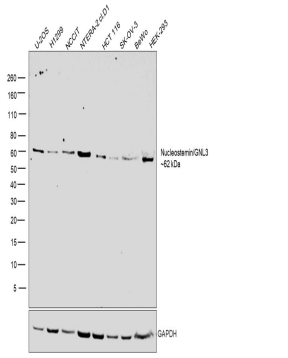
For Research Use Only. Not for use in diagnostic procedures. Not for resale without express authorization.

Product Images For Nucleostemin Recombinant Polyclonal Antibody (3HCLC)



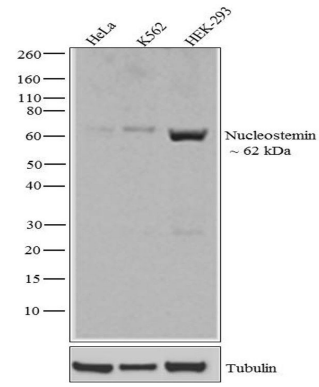
Nucleostemin Antibody (710188)

Antibody specificity was demonstrated by siRNA mediated knockdown of target protein. U-2 OS cells were transfected with Guanine nucleotide-binding protein-like 3 siRNA and a decrease in the signal intensity was observed in Western Blot application using Anti-Nucleostemin Recombinant Polyclonal Antibody (3HCLC) (Product # 710188). {KD}



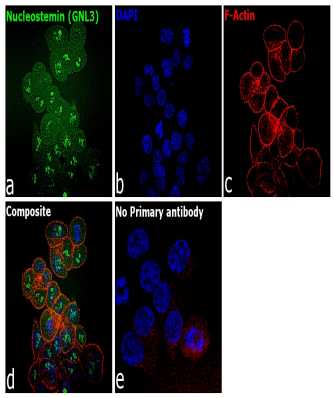
Nucleostemin Antibody (710188) in WB

Western blot was performed using Anti-Nucleostemin Recombinant Polyclonal Antibody (3HCLC) (Product # 710188) and a 62kDa band corresponding to Guanine nucleotide-binding protein-like 3 was observed across all the cell lines tested. Nuclear enriched extracts (30 µg lysate) of U-2 OS (Lane 1), H1299 (Lane 2), NCCIT (Lane 3), NTERA-2 cl.D1 (Lane 4), HCT 116 (Lane 5), SK-O-V3 (Lane 6), BeWo (Lane 7), HEK-293 (Lane 8) were electrophoresed using NuPAGE™ 4-12% Bis-Tris Protein Gel (Product # NP0322BOX). Resolved proteins were then transferred onto a nitrocellulose membrane (Product # IB23001) by iBlot® 2 Dry Blotting System (Product # IB21001). The blot was probed with the primary antibody (1 µg/mL) and detected by chemiluminescence with Goat anti-Rabbit IgG (Heavy Chain) Superclonal™ Recombinant Secondary Antibody, HRP (Product # A27036,1:20,000 dilution) using the iBright FL 1000 (Product # A32752). Chemiluminescent detection was performed using SuperSignal™ West Pico PLUS Chemiluminescent Substrate (Product # 34580).



Nucleostemin Antibody (710188) in WB

Western blot analysis of Nucleostemin was performed by loading 20 µg of HeLa (lane1), K562 (lane2) and HEK-293 (lane3) cell lysates using Novex®NuPAGE®4-12 % Bis-Tris gel (Product # NP0321BOX), XCell SureLock Electrophoresis System (Product # EI0002), Novex® Sharp Pre-Stained Protein Standard (Product # LC5800), and iBlot® Dry Blotting System (Product # IB21001). Proteins were transferred to a nitrocellulose membrane and blocked with 5 % skim milk for 1 hour at room temperature. Nucleostemin was detected at ~62 kDa using Nucleostemin Recombinant Rabbit Polyclonal Antibody (Product # 710188) at 0.5 µg-1 µg/mL in 2.5 % skim milk at 4°C overnight on a rocking platform. Goat anti-Rabbit IgG-HRP Secondary Antibody (Product # G-21234) at 1:5000 dilution was used and chemiluminescent detection was performed using Pierce™ ECL Western blotting Substrate (Product # 32106).



Nucleostemin Antibody (710188) in ICC/IF

Immunofluorescence analysis of Guanine nucleotide-binding protein-like 3 was performed using 70% confluent log phase U-2 OS cells. The cells were fixed with 4% paraformaldehyde for 10 minutes, permeabilized with 0.1% Triton™ X-100 for 15 minutes, and blocked with 2% BSA for 45 minutes at room temperature. The cells were labeled with Nucleostemin Recombinant Polyclonal Antibody (3HCLC) (Product # 710188) at 2 µg/mL in 0.1% BSA, incubated at 4 degree celsius overnight and then labeled with Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor Plus 488 (Product # A32790), (1:2000 dilution), for 45 minutes at room temperature (Panel a: Green). Nuclei (Panel b:Blue) were stained with Hoechst 33342 (Product # H1399). F-actin (Panel c: Red) was stained with Rhodamine Phalloidin (Product # R415, 1:300 dilution). Panel d represents the merged image showing nucleolar as well as nucleoplasmic localization. Panel e represents control cells with no primary antibody to assess background. The images were captured at 40X magnification in CellInsight CX7 LZR High-Content Screening (HCS) Platform (Product # CX7A1110LZR) and externally deconvoluted (D.Sage et al. / Methods 115 (2017) 28-41).

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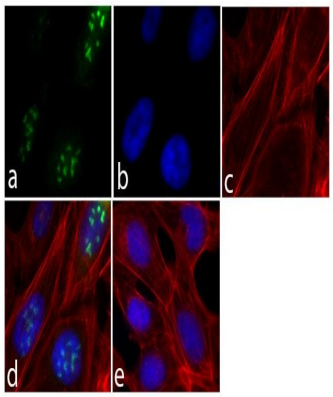
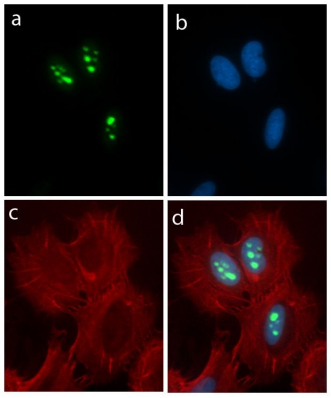


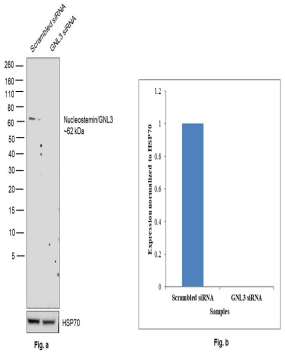
Nucleostemin Antibody (710188) in ICC/IF

Immunofluorescent analysis of Nucleostemin was done on 70% confluent log phase U2OS cells. The cells were fixed with 4% paraformaldehyde for 15 minutes; permeabilized with 0.25% Triton X-100 for 10 minutes followed by blocking with 5% BSA for 1 hour at room temperature. The cells were incubated with Nucleostemin Recombinant Rabbit Polyclonal Antibody (Product # 710188) at 2 µg-4 µg in 1% BSA and incubated for 3 hours at room temperature and then labeled with Alexa Fluor® 488 Goat anti-Rabbit IgG Secondary Antibody (Product # A-11008) at a dilution of 1: 400 for 30 minutes at room temperature (Panel a: green). Nuclei (Panel b: blue) were stained with SlowFade® Gold Antifade Mountant with DAPI (Product # S36938). F-actin (Panel c: red) was stained with Alexa Fluor® 594 Phalloidin (Product # A12381). Panel d is a merged image showing nucleolus localization of Nucleostemin. Panel e shows no primary antibody control. The images were captured at 20X magnification.



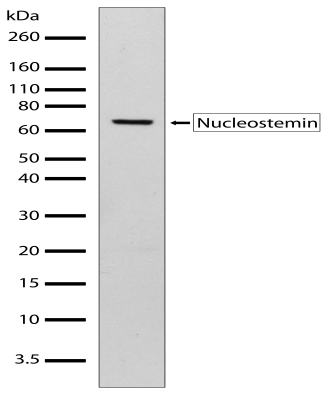
Nucleostemin Antibody (710188) in ICC/IF

Immunofluorescent analysis of Nucleostemin in HeLa cells using a Nucleostemin Recombinant Rabbit Polyclonal Antibody (Product # 710188) followed by detection using an Alexa Fluor 488-conjugated Goat anti-Rabbit secondary antibody (green) (Image A). Nuclei were stained using DAPI (Image B) and actin stained with Alexa Fluor 594 phalloidin (red) (image C). Image D is a composite image showing nuclear localization of nucleostemin.



Nucleostemin Antibody (710188) in WB

Knockdown of Guanine nucleotide-binding protein-like 3 was achieved by transfecting U-2 OS with Guanine nucleotide-binding protein-like 3 specific siRNAs (Silencer® select Product # s25421, s25422). Western blot analysis (Fig. a) was performed using Nuclear enriched extracts from the Guanine nucleotide-binding protein-like 3 knockdown cells (lane 2) and non-targeting scrambled siRNA transfected cells (lane 2). The blot was probed with Nucleostemin Recombinant Polyclonal Antibody (3HCLC) (Product # 710188, 1 µg/mL) and Goat anti-Rabbit IgG (Heavy Chain) Superclonal™ Recombinant Secondary Antibody, HRP (Product # A27036, 1:20,000 dilution). Densitometric analysis of this western blot is shown in histogram (Fig. b). The decrease in signal upon siRNA mediated knock down confirms that the antibody is specific to Guanine nucleotide-binding protein-like 3.



Nucleostemin Antibody (710188) in WB

Western blot analysis of Nucleostemin in HeLa whole cell extracts using a Nucleostemin Recombinant Rabbit Polyclonal Antibody (Product # 710188) at a dilution of 2 µg/mL. Samples were detected using chemiluminescence (ECL). Results show a band at ~62kDa.

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