Product Datasheet

Dicer knockout Mouse embryonic stem cells
NBP1-96751

Unit Size: 2 ml

Store in gas phase of liquid nitrogen.

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Publications: 5

Protocols, Publications, Related Products, Reviews, Research Tools and Images at:
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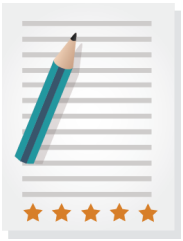
Updated 10/23/2024 v.20.1



NBP1-96751

Dicer knockout Mouse embryonic stem cells

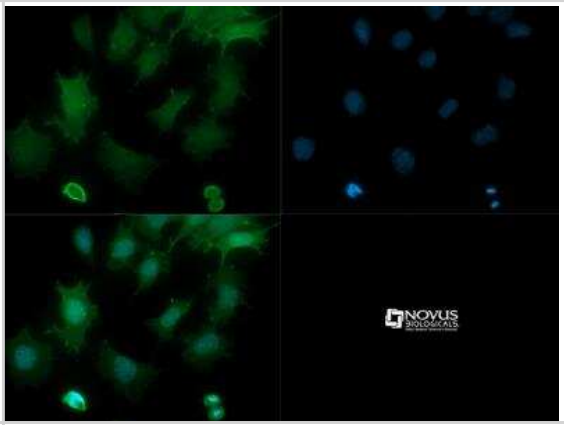
Product Information	
Unit Size	2 ml
Concentration	Please see the protocols for proper use of this product. If no protocol is available, contact technical services for assistance.
Storage	Store in gas phase of liquid nitrogen.
Buffer	Cells are supplied in 2 ml quantities (about 1x10 ^ 6 cells/ml) in freezing media (60% DMEM, 20% FBS, 20% DMSO)

Product Description	
Gene ID	23405
Gene Symbol	DICER1

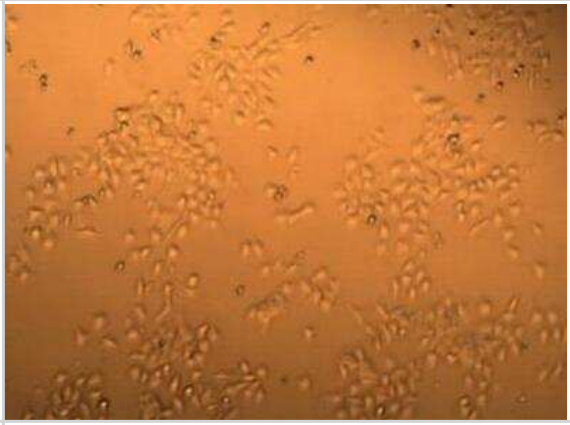
Product Application Details	
Application Notes	Please see Protocols and Procedures for culturing conditions.

Images

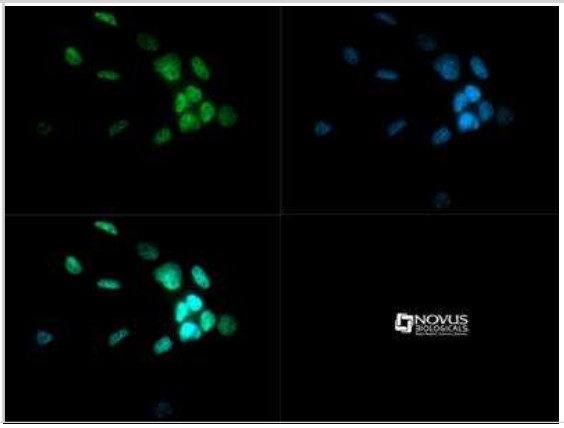
Immunocytochemistry/Immunofluorescence: Dicer knockout Mouse embryonic stem cells [NBP1-96751] - SSEA1 antibody (NB100-1831) was tested in Dicer knockout Mouse embryonic stem cells with DyLight 488 (green). Nuclei were counterstained with DAPI (blue).



Immunohistochemistry: Dicer knockout Mouse embryonic stem cells [NBP1-96751] - Brightfield Image of Dicer knock out Mouse embryonic stem cell line colonies growing directly on a gelatinized tissue culture flask.



Immunocytochemistry/Immunofluorescence: Dicer knockout Mouse embryonic stem cells [NBP1-96751] - Nanog antibody (NB100-58842) was tested in Dicer knockout Mouse embryonic stem cells with DyLight 488 (green). Nuclei were counterstained with DAPI (blue).



Publications

Cinghu S, Yang P, Kosak JP et al. Intragenic Enhancers Attenuate Host Gene Expression. Mol. Cell. 2017-10-05 [PMID: 28985501] (Mouse)

Umemura Y, Koike N, Ohashi M et al. Involvement of posttranscriptional regulation of Clock in the emergence of circadian clock oscillation during mouse development Proc. Natl. Acad. Sci. U.S.A. 2017-08-21 [PMID: 28827343] (Mouse)

Krawczynski K, Najmula J, Bauersachs S, Kaczmarek MM. MicroRNAome of porcine conceptuses and trophoblasts: expression profile of micrornas and their potential to regulate genes crucial for establishment of pregnancy. Biol Reprod. [PMID: 25472924]

Estruch SB, Graham SA, Deriziotis P, Fisher SE The language-related transcription factor FOXP2 is post-translationally modified with small ubiquitin-like modifiers. Sci Rep 2016-02-12 [PMID: 26867680] (WB)

Babiarz JE, Ruby JG, Wang Y, Bartel DP, Blelloch R. Mouse ES cells express endogenous shRNAs, siRNAs, and other Microprocessor-independent, Dicer-dependent small RNAs. Genes Dev. 22(20):2773-85. 2008-10-15 [PMID: 18923076] (Mouse)

Procedures

Protocol Specific for Dicer knockout Mouse embryonic stem cells (NBP1-96751)
Dicer knockout Mouse embryonic stem cells:
Protocol Specific for Dicer knockout Mouse embryonic stem cells

Growing Dicer mouse ES cells

This protocol is written for growing cells in T25 tissue culture flasks, please make changes accordingly for flasks of different sizes. ES cells are routinely cultured in ES medium in the presence of LIF on a mitotically inactivated MEF feeder layer grown on gelatin.

1. Media:
ESL1000 for ES cells:
DMEM-Hi glucose 425 ml (Caisson Labs, DML10-500ML)
FBS 75 ml (biowest, US1520)
100 X non-essential amino acid 5 ml (Millipore EmbryoMax(R) TMS-001-C)
200 mM L-Glutamine 5 ml - (Sigma G7513)
100% beta-mercaptoethanol (100X for ES cells) 5 ml (Millipore EmbryoMax(R) ES-007-E)
1000 U/ml Lif 0.5 ml (Millipore ESGRO(R) ESG1106)
MEF for embryonic fibroblasts:
DMEM-Hi glucose 450 ml (Caisson Labs, DML10-500ML)
FBS 50 ml (biowest, US1520)
100 X non-essential amino acid 5 ml (Millipore EmbryoMax(R) TMS-001-C)
200 mM L-Glutamine 5 ml - (Sigma G7513)
100% beta-mercaptoethanol (100X for ES cells) 5 ml (Millipore EmbryoMax(R) ES-007-E)

2. Preparation of gelatin coated tissue culture flasks:
To make gelatinized flasks, distribute a thin layer (about 2ml per T25 flask) of Millipore EmbryoMax(R) Ultrapure water with 0.1% gelatin (catalog# ES-006-B) onto a T25 tissue culture flask and incubate at 37 degrees Celsius for 15 minutes. Remove the gelatin solution and set aside.

3. MEF feeder flasks:
Maintain MEF cells in MEF media for embryonic fibroblasts. The thawed MEF cells can be grown and maintained in a regular T25 tissue culture flask and when confluent, transferred to a T150 flask. Gelatin is not needed for the culture MEF feeder cells.
a. Mitotic inactivation (Mitomycin C treatment) for preparation of ES feeder layers:
At confluence, Mitomycin C is used as a treatment to halt cell division. Use the procedure below to prepare fresh MEF feeder layers.
*Plate mitomycin C treated MEFs in a gelatinized T25 at least one day but not more than 1 week before plating ES cells on the feeder.
3.1 To one T150 tissue culture flask of confluent MEF cells: remove regular growth medium and add 40 ml of fresh MEF medium containing 40ul of Mitomycin C (Sigma, catalog# M4287-2MG) and incubate overnight.
3.2 Remove mitomycin C containing medium and wash twice with PBS, trypsinize, resuspend and replate by dispensing 2ml of MEF cell split into desired number of T25 gelatinized flasks. Note that for this step, a split ratio of about 1:1 or a bit less should be used. The reasoning behind the 1:1 split ratio is to achieve the best feeder cell density. The cells should almost completely cover the bottom of the flask but with enough space left for the ES cell colonies to spread out a bit. As it directly affects the growth of the ES cells, feeder layer quality is extremely important.

4. Thawing ES cells from -80 C or Liquid N2:
Thaw a tube of 2 X 10^6 ES cells in 37 C water bath for 1-2 minutes. During this time, prepare a 15-ml tube, add 10 ml warm ESL1000 media; then pipette out the thawed cells and mix with warm media in the 15-ml tube by gently pipetting up and down a few times. Spin down cells at 1000 rpm for 5 minutes. Aspirate off the media carefully without touching cell pellets, add 8 ml fresh ESL1000 media, pipette up and down a few times, plate onto a T25 tissue culture flask with MEF feeder cells grown on gelatin.

5. Passaging cells:
Aspirate off the media, wash once with Hank's buffered saline or PBS with 1 mM EDTA, add 2ml-0.1% Trypsin to a

T25 flask, incubate at 37 C for 5 minutes. Add 2 ml ESL1000 media to the flask, pipette to dislodge cells and plate onto the T25 tissue culture flasks containing MEF feeder cells grown on gelatin. Medium is changed every day and cells are usually split at a 1 to 4 or 5 ratio in 2 days.

6. Freezing cells:
Freeze cells in 1 part of fresh media and 1 part of 2 X freezing media (60% DMEM, 20% FBS, 20% DMSO). Use cryo safe tube. Save tubes in a Styrofoam box at -80 C. For long term storage, move them to liquid nitrogen a few days later.

Novus Biologicals USA
10730 E. Briarwood Avenue
Centennial, CO 80112
USA
Phone: 303.730.1950
Toll Free: 1.888.506.6887
Fax: 303.730.1966
nb-customerservice@bio-techne.com

Bio-Techne Canada
21 Canmotor Ave
Toronto, ON M8Z 4E6
Canada
Phone: 905.827.6400
Toll Free: 855.668.8722
Fax: 905.827.6402
canada.inquires@bio-techne.com

Bio-Techne Ltd
19 Barton Lane
Abingdon Science Park
Abingdon, OX14 3NB, United Kingdom
Phone: (44) (0) 1235 529449
Free Phone: 0800 37 34 15
Fax: (44) (0) 1235 533420
info.EMEA@bio-techne.com

General Contact Information
www.novusbio.com
Technical Support: nb-technical@bio-techne.com
Orders: nb-customerservice@bio-techne.com
General: novus@novusbio.com

Products Related to NBP1-96751

DVE00	VEGF [HRP]
NBP1-97435	Dicer knockout Mouse embryonic stem cells Lysate
NB600-302	c-Myc Antibody (9E10) - BSA Free
NB500-171	Histone H3 Antibody - BSA Free

Limitations

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