Product Datasheet

DGCR8 knockout Mouse embryonic stem cells
NBA1-19349

Unit Size: 2 ml

Store in gas phase of liquid nitrogen.

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Publications: 13

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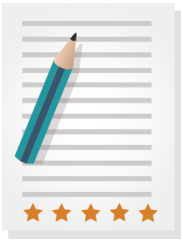
Updated 10/23/2024 v.20.1



NBA1-19349

DGCR8 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

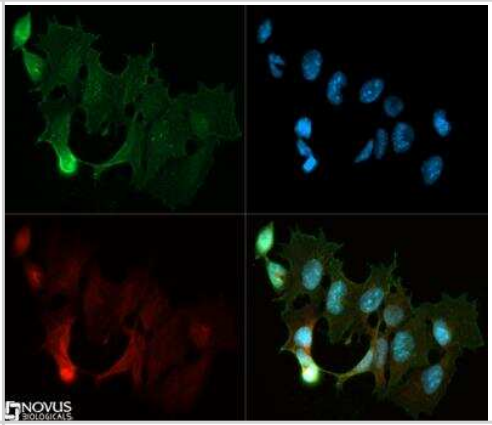
Gene ID	54487
Gene Symbol	DGCR8
Species	Mouse

Product Application Details

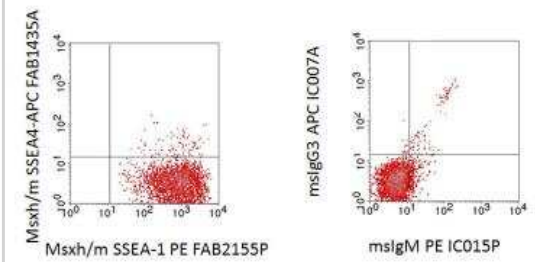
Applications	Flow Cytometry, Immunocytochemistry/ Immunofluorescence, In vitro assay, In vivo assay
Recommended Dilutions	Flow Cytometry, Immunocytochemistry/ Immunofluorescence, In vitro assay, In vivo assay reported in scientific literature
Application Notes	Recommended Media: StemXVivo Mouse Pluripotent Stem Cell Media Kit (R&D Systems, Cat# CCM025). Please see Protocol for aditional culturing conditions.

Images

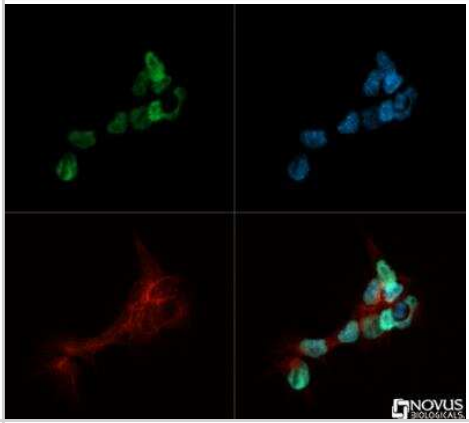
Immunocytochemistry/Immunofluorescence: DGCR8 knockout Mouse embryonic stem cells [NBA1-19349] - SSEA1 antibody (NB100-1831) was tested in with DyLight 488 (green). Nuclei and beta-tubulin were counterstained with DAPI (blue) and DyLight 550 (red).



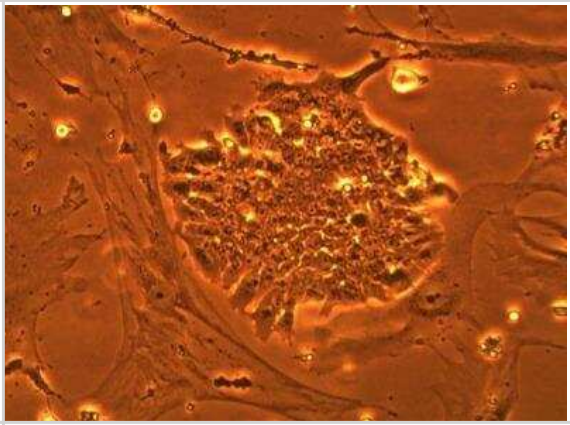
Flow Cytometry: DGCR8 knockout Mouse embryonic stem cells [NBA1-19349] - DCGR8 mouse embryonic stem cells were cultured for 5 passages in StemXVivo Mouse Pluripotent Stem Cell Media (R&D Systems, Catalog # CCM025). At passage 5, cells were harvested and analyzed for pluripotent markers by flow cytometry. Expression of SSEA-1 and SSEA-4 were detected using PE-conjugated Mouse Anti-SSEA-1 Monoclonal Antibody (R&D Systems, Catalog # FAB2155P) and APC-conjugated Mouse Anti-SSEA-4 Monoclonal Antibody (R&D Systems, Catalog # FAB1435A). Quadrant lines were drawn based on matched isotype controls.



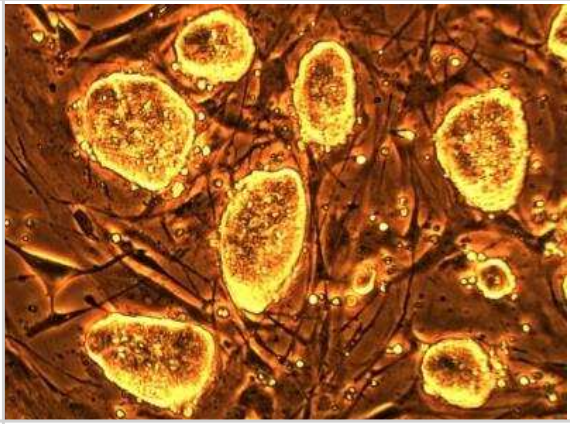
Immunocytochemistry/Immunofluorescence: DGCR8 knockout Mouse embryonic stem cells [NBA1-19349] - Nanog antibody (NB100-58842) was tested in with DyLight 488 (green). Nuclei and alpha-tubulin were counterstained with DAPI (blue) and DyLight 550 (red).



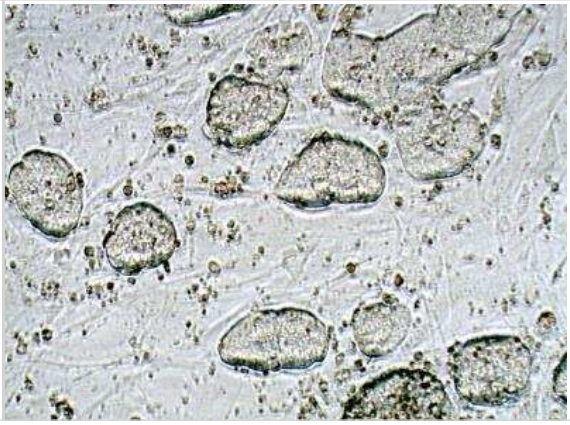
In vitro assay: DGCR8 knockout Mouse embryonic stem cells [NBA1-19349] - Brightfield Image of DGCR8 knockout Mouse embryonic stem cell [NBA1-19349] colonies growing directly on a gelatinized tissue culture flask.



In vitro assay: DGCR8 knockout Mouse embryonic stem cells [NBA1-19349] - Brightfield Image of DGCR8 knockout Mouse embryonic stem cell [NBA1-19349] colonies growing on a Mitomycin C MEF cell feeder layer.



In vitro assay: DGCR8 knockout Mouse embryonic stem cells [NBA1-19349] - Brightfield Image of DGCR8 -/- ES cell colonies growing on a gamma-irradiated MEF cell feeder layer.



Publications

Vardapour R, Kehl T, Kneitz S et al. The DGCR8 E518K mutation found in Wilms tumors leads to a partial miRNA processing defect that alters gene expression patterns and biological processes Carcinogenesis 2021-12-17 [PMID: 34919667]

Majumder S, Ren L, Pushpakumar S, Sen U MicroRNA-deficient mouse embryonic stem cells acquire a functional interferon response Elife 2019-04-23 [PMID: 31012846] (WB, Mouse)

Freimer JW, Hu TJ, Blelloch R. Decoupling the impact of microRNAs on translational repression versus RNA degradation in embryonic stem cells Elife 2018-07-25 [PMID: 30044225] (In vitro, 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]

Macias S, Cordiner RA, Gautier P et al. DGCR8 Acts as an Adaptor for the Exosome Complex to Degrade Double-Stranded Structured RNAs. Mol Cell 2015-12-17 [PMID: 26687677] (WB)

Lei Z, van Mil A, van de Vrugt AM. Dgcr8 is Indispensable for Cardiac Lineage Specification in Embryonic Stem Cells Stem CellResearch & Therapy. 2015-01-15 (In vitro)

Details:
DGCR8 knockout Mouse embryonic stem cells and v6.5 Mouse embryonic stem cells used for experiments involving in vito cardiac differentiation. Dgcr8 KO mouse embryonic stem cells alongwith v6.5 WT controls were also characterized for the loss of Dgcr8 protein (in the Dgcr8 KO-ESCs) with WB, Genotyping, ICC-IF and RNA profiling (Figure 1).

Ciaudo C, Jay F, Okamoto I et al. RNAi-Dependent and Independent Control of LINE1 Accumulation and Mobility in Mouse Embryonic Stem Cells. PLoS Genet. 2013-11-01 [PMID: 24244175]

Jiang K, Ren C, Nair VD. MicroRNA-137 represses Klf4 and Tbx3 during differentiation of mouse embryonic stem cells. Stem Cell Res. 2013-09-13 [PMID: 24084696]

Heras SR, Macias S, Plass M et al. The Microprocessor controls the activity of mammalian retrotransposons. Nat Struct Mol Biol. 2013-09-01 [PMID: 23995758]

Stankiewicz TR, Schroeder EK, Kelsey NA et al. C-terminal binding proteins are essential pro-survival factors that undergo caspase-dependent downregulation during neuronal apoptosis. Mol Cell Neurosci 2013-07-13 [PMID: 23859824]

Macias S, Plass M, Stajuda A et al. DGCR8 HITS-CLIP reveals novel functions for the Microprocessor Nat Struct Mol Biol 2012-08-01 [PMID: 22796965]

Wang et al. DGCR8 is essential for microRNA biogenesis silencing of embryonic stem cell self-renewal. Nat Genet; 39(3):380-5. 2007-03-01 [PMID: 17259983] (In vitro)

More publications at http://www.novusbio.com/NBA1-19349

Procedures

Protocol specific for DGCR8 knockout Mouse embryonic stem cells (NBA1-19349)

Protocol Specific for DGCR8 knockout Mouse embryonic stem cells

Growing DGCR8 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)
10 ng/ml LIF (R&D Systems 8878-LF)
** or use StemXVivo Mouse Pluripotent Stem Cell Media Kit instead (R&D Systems, cat# CCM025)

MEF Media 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 TrypLE (Gibco) to a T25 flask, incubate at 37 C for 1-2 minutes. Add 2 ml ESL1000 media to the flask, pipette to dislodge cells, spin at 1000rpm for 5 minutes 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 5-6 x10^4/cm^2.

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.

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NBP2-32887-0.1mg	Ornithine Decarboxylase Antibody (ODC1/485)

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