Product Datasheet

v6.5 Mouse embryonic stem cells
NBP1-41162

Unit Size: 2 ml

Store in gas phase of liquid nitrogen.

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Publications: 29

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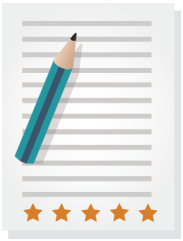
Updated 6/29/2023 v.20.1



NBP1-41162

v6.5 Mouse embryonic stem cells

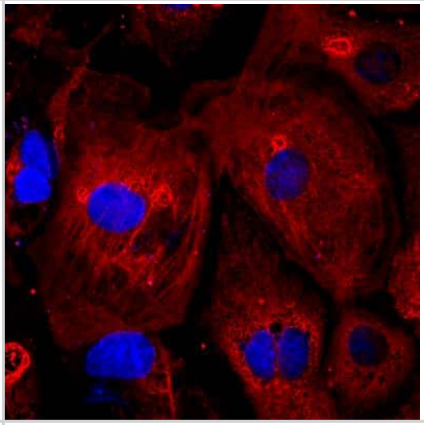
Product Information	
Unit Size	2 ml
Concentration	Please see the protocols for proper use of this product. If no protocol is available, contact technical services for assistance.
Storage	Store in gas phase of liquid nitrogen.
Buffer	Cells are supplied in 2 ml quantities (about 1x10 ^ 6 cells/ml) in Freezing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

Product Description	
Species	Mouse
Specificity/Sensitivity	v6.5 Mouse embryonic stem cells from a male mouse embryo.
Notes	Shipping is on dry ice. Proper long-term strorage is gas phase of liquid nitrogen.

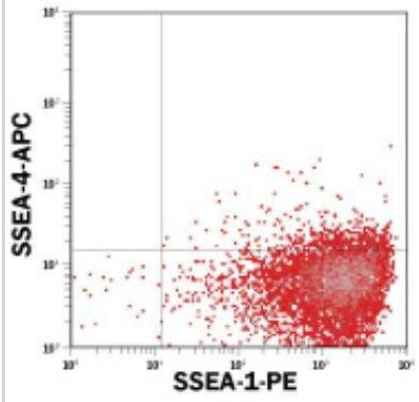
Product Application Details	
Applications	In vitro assay
Recommended Dilutions	In vitro assay
Application Notes	Recommended Media: StemXVivo Mouse Pluripotent Stem Cell Media Kit (R&D Systems, Cat# CCM025). Please see Protocol for additional culturing conditions.

Images

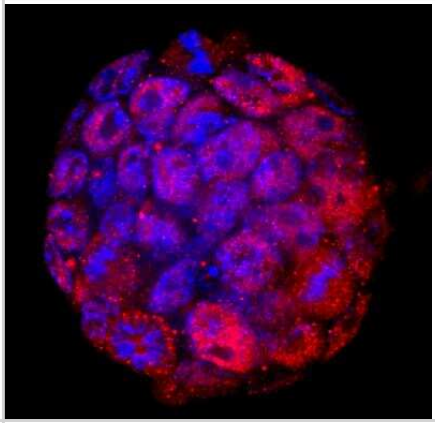
Immunocytochemistry: v6.5 Mouse embryonic stem cells [NBP1-41162] - Embryonic Stem Cells Grown in Mouse Pluripotent Stem Cell Media Are Capable of Tri-Lineage Differentiation. v6.5 mouse embryonic stem cells were cultured in complete StemXVivo Mouse Pluripotent Stem Cell Media. For differentiation, cells were cultured as embryoid bodies for 5 days, plated onto Cultrex BME-coated plates (R&D Systems, Catalog # 3434-005-02) for an additional 5-10 days. Expression of the mesoderm marker, Smooth Muscle Actin, was detected in 15 day cells using Mouse Anti-Smooth Muscle Actin monoclonal antibody (R&D Systems, Catalog # MAB1420). The nuclei were counterstained with DAPI.



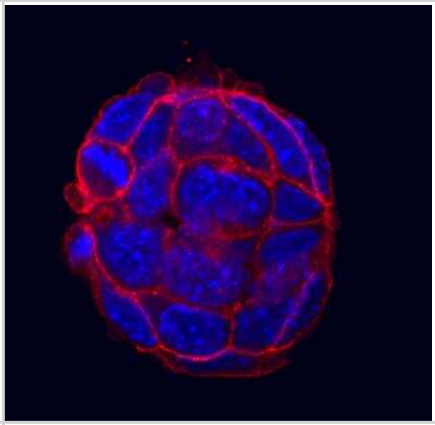
Flow Cytometry: v6.5 Mouse embryonic stem cells [NBP1-41162] - Embryonic Stem Cells Grown in Mouse Pluripotent Stem Cell Media Express the Pluripotency Stem Cell Marker SSEA-1 and Lack SSEA-4. v6.5 mouse embryonic stem cells (Novus Biologicals, NBP1-41162) were cultured in complete StemXVivo Mouse Pluripotent Stem Cell media. 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. The nuclei were counterstained with DAPI.



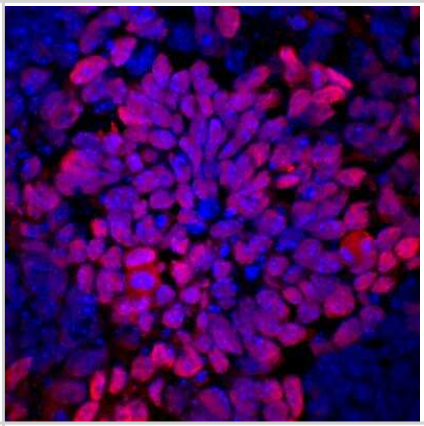
Immunocytochemistry: v6.5 Mouse embryonic stem cells [NBP1-41162] - Embryonic Stem Cells Grown in Mouse Pluripotent Stem Cell Media Express the Pluripotency Stem Cell Marker Oct-3/4. v6.5 mouse embryonic stem cells (Novus Biologicals, NBP1-41162) were cultured in complete StemXVivo Mouse Pluripotent Stem Cell media. Expression of Oct-3/4 was detected using Rat Anti-Human/Mouse Oct-3/4 Monoclonal Antibody (R&D Systems, Catalog # MAB1759) followed by NorthernLights (NL)557-conjugated Goat Anti-Rat Secondary Antibody (R&D Systems, Catalog # NL013). The nuclei were counterstained with DAPI.



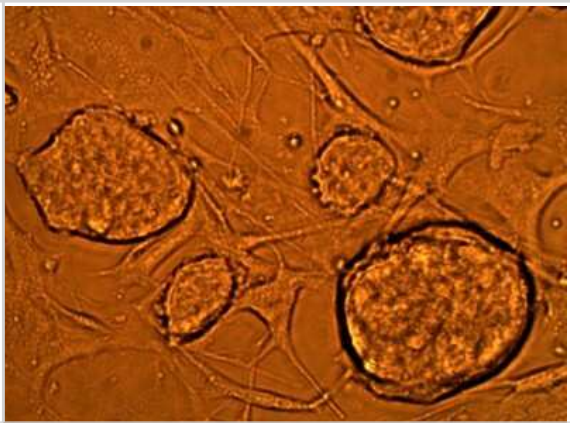
Immunocytochemistry: v6.5 Mouse embryonic stem cells [NBP1-41162] - Embryonic Stem Cells Grown in Mouse Pluripotent Stem Cell Media Express the Pluripotency Stem Cell Marker Alkaline Phosphatase. v6.5 mouse embryonic stem cells (Novus Biologicals, NBP1-41162) were cultured in complete StemXVivo Mouse Pluripotent Stem Cell media. Expression of Alkaline Phosphatase was detected using Goat Anti-Mouse Alkaline Phosphatase Affinity Purified Polyclonal Antibody (R&D Systems, Catalog # AF2910) followed by NL557-conjugated Donkey Anti-Goat Secondary Antibody (R&D Systems, Catalog # NL001). The nuclei were counterstained with DAPI.



Immunocytochemistry: v6.5 Mouse embryonic stem cells [NBP1-41162] - Embryonic Stem Cells Grown in Mouse Pluripotent Stem Cell Media Are Capable of Tri-Lineage Differentiation. v6.5 mouse embryonic stem cells were cultured in complete StemXVivo Mouse Pluripotent Stem Cell Media. For differentiation, cells were cultured as embryoid bodies for 5 days, plated onto Cultrex BME-coated plates (R&D Systems, Catalog # 3434-005-02) for an additional 5-10 days. Expression of the ectoderm marker, SOX1, was detected in day 10 cells using Goat Anti-SOX1 Affinity Purified Polyclonal Antibody (R&D Systems, Catalog # AF3369) followed by NorthernLights (NL)557-conjugated Donkey Anti-Goat Secondary Antibody. The nuclei were counterstained with DAPI.

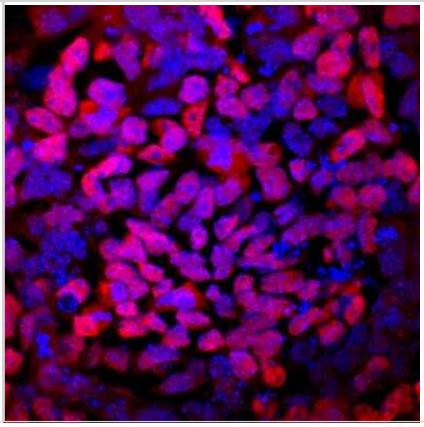


In vitro assay: v6.5 Mouse embryonic stem cells [NBP1-41162] - Brightfield Image of v6.5 Mouse embryonic stem cell [NBP1-41162] colonies growing on a Mitomycin C treated MEF cell feeder layer.



In vitro assay: v6.5 Mouse embryonic stem cells [NBP1-41162] - Brightfield Image of v6.5 Mouse embryonic stem cell [NBP1-41162] colonies growing directly on a gelatinized tissue culture flask.

Immunodiffusion: v6.5 Mouse embryonic stem cells [NBP1-41162] - Embryonic Stem Cells Grown in Mouse Pluripotent Stem Cell Media Are Capable of Tri-Lineage Differentiation. v6.5 mouse embryonic stem cells were cultured in complete StemXVivo Mouse Pluripotent Stem Cell Media. For differentiation, cells were cultured as embryoid bodies for 5 days, plated onto Cultrex BME-coated plates (R&D Systems, Catalog # 3434-005-02) for an additional 5-10 days. Expression of the endoderm marker, SOX17, was detected in 10 day cells using Goat Anti-SOX17 Affinity Purified Polyclonal Antibody (R&D Systems, Catalog # AF1924) followed by NL557-conjugated Donkey Anti-Goat Secondary Antibody. The nuclei were counterstained with DAPI.



Publications

Tao F, Rhonda E, He X et al. An optimized chromatin immunoprecipitation protocol using Staph-seq for analyzing genome-wide protein-DNA interactions STAR Protocols 2022-12-01 [PMID: 36595937]

Tan K, Wilkinson MF Regulation of both transcription and RNA turnover contribute to germline specification Nucleic acids research 2022-07-22 [PMID: 35776114] (Cell Culture, Cell-based ELISA, Mouse)

Details:
Mouse ESCs were induced to differentiate into EpiLCs and PGCLCs

Tao F, Soffers J, Hu D et al. beta-Catenin and Associated Proteins Regulate Lineage Differentiation in Ground State Mouse Embryonic Stem Cells Stem Cell Reports 2020-01-01 [PMID: 32822591]

Li F, Huangyang P, Burrows M et al. FBP1 loss disrupts liver metabolism and promotes tumorigenesis through a hepatic stellate cell senescence secretome Nat. Cell Biol. 2020-05-04 [PMID: 32367049] (Mouse)

Yang L, Liu X, Song L et al. Melatonin restores the pluripotency of long-term-cultured embryonic stem cells through melatonin receptor-dependent m6A RNA regulation J. Pineal Res. 2020-05-16 [PMID: 32415999]

Gamache J, Benzow K, Forster C et al. Factors other than hTau overexpression that contribute to tauopathy-like phenotype in rTg4510 mice Nat Commun. 2019-06-05 [PMID: 31171783] (Mouse)

Mumbach MR, Satpathy AT, Boyle EA et al. Enhancer connectome in primary human cells reveals target genes of disease-associated DNA elements Nat Genet. [PMID: 28945252] (In vitro, Mouse)

Chen Xingqi, Litzenburger Ulrike M, Wei Yuning et al. Joint single-cell DNA accessibility and protein epitope profiling reveals environmental regulation of epigenomic heterogeneity. Frontiers in Physiology 2018-11-02 [PMID: 30389926] (Mouse)

Potapova TA, Unruh JR, Yu Z et al. Superresolution microscopy reveals linkages between ribosomal DNA on heterologous chromosomes J. Cell Biol. 2019-07-03 [PMID: 31270138]

Kim JH, Rege M, Valeri J et al. LADL: light-activated dynamic looping for endogenous gene expression control Nat Methods. 2019-07-01 [PMID: 31235883]

Sul OJ, Rajasekaran M, Park HJ et al. Reversible Disruption of Specific Transcription Factor-DNA Interactions Using CRISPR/Cas9 Mol. Cell 2019-05-02 [PMID: 31051141] (Mouse)

Sans A, Bonnafous S, Rousseau D et al. HiChIRP reveals RNA-associated chromosome conformation Nat. Methods 2019-06-01 [PMID: 31133759] (Mouse)

More publications at http://www.novusbio.com/NBP1-41162

Procedures

Protocol specific for v6.5 Mouse embryonic stem cells (NBP1-41162)

Protocol Specific for v6.5 Mouse embryonic stem cells

Growing v6.5 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 distilled water with 0.1% gelatin (Sigma) onto a T25 tissue culture flask and incubate at 37 degrees Celsius for 1 hour. Remove the gelatin solution and begin plating the stem cells.

3. MEF feeder flasks:
According to the Culture of Animal Cells textbook by R. Ian Freshney, MEF cells used as a feeder layer should not exceed 6 passages prior to treating with Mitomycin C.

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

10ml warm ESL1000 media. Mix the thawed cells with warm media in the 15-ml tube by adding the thawed cells to the side of the conical tube of warm media just above the buffer level and rotate the conical tube slowly and pipette slowly so the cells fall into the media gradually. Spin down cells at 1000 rpm for 5 minutes. Aspirate off the media carefully without touching cell pellets, add 8 ml fresh ESL1000 media and gently re-suspend the pellet, plate onto a T25 tissue culture flask with MEF feeder cells grown on gelatin.

5. Passaging cells:
After cells settle down (in 3-5 days), remove the media and replace with fresh ESL 1000 media. At 70-80% confluency, 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 and transfer to a sterile tube. Gently pellet the cells and reuspend in media, transfer approximately 0.25 x 10^6 cells per well of a 6 well plate containing 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

Limitations
This product is for research use only and is not approved for use in humans or in clinical diagnosis. Support products are guaranteed for 6 months from date of receipt.

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