

PRODUCT DESCRIPTION

STEMcircles™-LGNSO provide a non-viral, non-integrating approach to reprogramming somatic cells to induced pluripotent stem cells (iPSCs)¹. STEMcircles™-LGNSO plasmid DNA encodes the genes for four factors capable of reprogramming somatic cells: LIN28, NANOG, SOX2 and OCT3/4, as well as a GFP reporter. Minicircle vectors afford higher transfection efficiencies and more persistent expression compared to regular plasmid-based reprogramming techniques.^{2,3}

Patent pending.

COMPONENTS

05820 STEMcircles™-LGNSO 100µg

Contains:

- Purified DNA in Tris-EDTA buffer, sterility tested
- Concentration = 1 mg/mL

STABILITY AND STORAGE

This product has been aseptically manufactured.

Storage at -20°C to -80°C or below is recommended upon receipt. Before use, product can be thawed and aseptically aliquoted into convenient amounts and refrozen. Short-term storage of aliquots (up to two weeks) at 2 - 8°C is acceptable. Avoid multiple freeze thaw cycles.

DIRECTIONS FOR USE

Users should refer to the publications^{1,4} for directions for use. STEMcircles™-LGNSO plasmid DNA must be electroporated or transfected into the starting cells using protocols specifically optimized for that cell type.

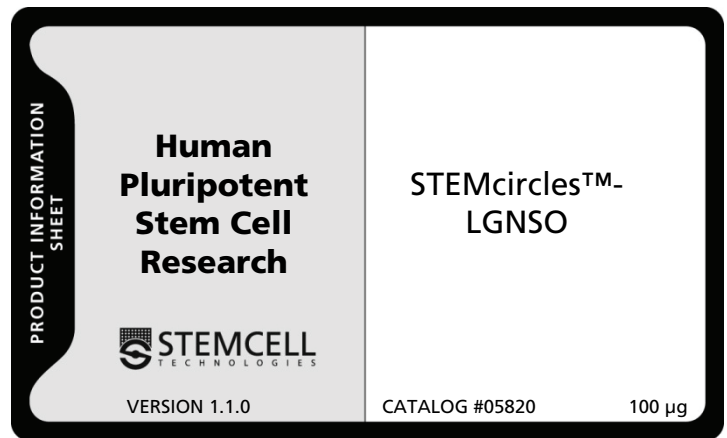
NOTES

Optimization of the transfection step will be necessary and will vary depending on the starting cell population. Transfection efficiency is generally dependent on cell number, amount of DNA and amount of transfection reagent. Users should follow instructions provided with their chosen reagent and optimize their protocol by varying one parameter at a time, keeping other parameters constant. For guidance on use with human neonatal fibroblasts and human adipose stem cells, please refer to the publications^{1,4}.

Note: STEMcircles™-LGNSO has been optimized for reprogramming human adipose stem cells and human neonatal fibroblasts (low efficiency). STEMcircles™-LGNSO has not yet been successful with reprogramming human dermal fibroblasts derived from adult sources⁴.

In cells that are difficult to transfect, access to an automated cell sorter may be necessary to select transfected cells to facilitate the calculation of the frequency of reprogramming.

Several publications describe morphological and functional criteria by which one can identify fully reprogrammed bona fide iPSCs^{5,6}.



STEMcircles™-LGNSO has been functionally validated by transiently transfecting HEK 293 cells with the DNA and observing GFP expression. Expression of LIN28, NANOG, SOX2 AND OCT3/4 were also confirmed in transfected HEK 293 cells at both the protein and RNA level by Western blot analysis and RT-PCR.

RELATED PRODUCTS

PRODUCT	CATALOG #
mTeSR™1	05850/05870/05875
TeSR™2	05860/05880
AggreWell™400 plates	27845/27945
AggreWell™800 plates	27865/27965
AggreWell™400Ex plates	27840/27940
mFeSR™	05855/05854
CryoStor™ CS10	07930
ACCUASE®	07920
Dispase (1 mg/mL)	07923
Anti-Oct 3/4 antibody	01550/01551
Anti-SSEA-1 antibody	01552
Anti-SSEA-3 antibody	01553
Anti-SSEA-4 antibody	01554
Anti-TRA-1-60 antibody	01555
Anti-TRA-1-81 antibody	01556

Refer to Material Safety Data Sheet for more information.

This product is for internal non-commercial research use only. No rights are conveyed to modify or clone the gene encoding fluorescent protein contained in this product. The right to use this fluorescent protein specifically excludes the right to validate or screen compounds. For information on commercial licensing, contact Evrogen Licensing Department, email: license@evrogen.com."

REFERENCES

1. Jia F.J., Wilson, K., Sun, N., Gupta,D.M.,, Huang, M, Li, H., Panetta, N.J., Chen, Z.Y., Robbins, R.C., Kay, M.A., Longaker, M.T., Wu, J.C., 2010. A non-viral minicircle vector for deriving human iPS cells, *Nature Methods* 7(3): 197-9.
2. Chen, Z.Y., He, C.Y., Ehrhardt, A., Kay, M.A., 2003. Minicircle DNA vectors devoid of bacterial DNA result in persistent and high-level transgene expression *in vivo*. *Molecular Therapeutics*. 8(3):495-500.
3. Chen, Z.Y., He, C.Y., Kay, M.A., 2005. Improved production and purification of minicircle DNA free of plasmid bacterial sequences and capable of persistent transgene expression *in vivo*. *Human Gene Therapy*. 16:126-131.
4. Narsinh KH, Jia F, Robbins RC, Kay MA, Longaker MT, Wu JC. 2011. Generation of adult human induced pluripotent stem cells using nonviral minicircle DNA vectors. *Nature Protocols*. Jan;6(1):78-88.
5. Chan E.M., Ratanasirinrawoot, S., Park, I.H., Manos, P.D., Loh, Y.H., Huo, H., Miller, J.D., Hartung, O., Rho, J., Ince, T.A., Daley, G.Q., Schlaeger, T.M., 2009. Live cell imaging distinguishes bona fide human iPS cells from partially reprogrammed cells. *Nature Biotechnology*: 27(11): 1033-77.
6. Maherali, N. and Hochedlinger, K., 2008. Guidelines and techniques for the generation of induced pluripotent stem cells. *Cell Stem Cell* 3: 595-605.