transfection genespin DATA SHEET

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> Transfection Kit

cat. no.	*amount*	*note*
STS-TK 1	1ml	10X solution,
STS-TK 1x4	4x1ml	10X solution,

GeneSpin developed StoS Transfection KIT, a powerful transfection tool that ensures effective and reproducible transfection of both human and mouse cell lines, as well as primary cells, with low toxicity. StoS Transfection KIT is provided in a 10x solution.

DNA transfected cell lines using StoS Transfection Kit

CELL	DESCRIPTION	DNA/StoS Tranfection Kit RATIO	Real % GFP or LacZ positive
COS1	monkey kidney cells	1:4	33%
HaCat	human keratinocyte cells	1:4	20%
HCTwt	human colorectal carcinoma	1:4	40%
HT1080	human fibrosarcoma	1:4	10%
SAOS	human primary osteogenic sarcoma	1:4	40%
U2OS	human bone osteosarcoma	1:4	10%
NIH3T3	murine embryonic fibroblasts	1:4	40%
HeLa	human cervix epitheloid carcinoma cells	1:5	26%
MCF-7	human breast adenocarcinoma cells	1:4	13%
HepG2	human hepatocarcinoma cells	1:4	23%
K562	human chronic myelogenous leukemia	1:3	10%
MEF	primary mouse skin fibroblast (2nd passage)	1:4	30%
MEF	primary mouse skin fibroblast (10th passage)	1:4	20%

Storage

StoS Transfection KIT is shipped at Room Temperature and should be stored at 4°C; protect from direct light. StoS Transfection KIT is stable at least for 6 months.

FOR RESEARCH USE ONLY

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> Transfection Kit -protocol

CELLS SEEDING

Seed cells one day prior to transfection. For the number of cells to be seeded in different vessels, please, refer to the table

Suggested number of cells

Cell Culture vessel	# cells to be seeded per well	Volume of Medium per well
96-well plate	$1.5x10^4$ - $2.5x10^4$	0.1-0.2ml
48-well plate	$3x10^4$ - $5x10^4$	0.25-0.5ml
24-well plate	$6x10^4$ -$1x10^5$	0.5-1.0ml
12-well plate	$1.2x10^5$ - $2x10^5$	1.0-2.0ml
6-well plate	$2x10^5$ - $4x10^5$	2.0-4.0ml

The following protocol is intended for transfecting cells in 6-well plates (for different cell culture formats, please refer to the table below) with a 1:3 DNA/StoS Transfection Kit 10x ratio.
Remember that the DNA /StoS Transfection Kit 10x ratio depends on the cell line.
Change medium to cells before preparing the Transfection Mix: add 1ml of Complete Medium to each well.
For each well, diluite 2ug of DNA into 10ul of Serum-free Medium (Mix A), mix gently and spin down briefly.
For each well, diluite 6ul of StoS Transfection KIT 10x into 54ul of Serum-free Medium (Mix B) (StoS Transfection KIT final concentration is 1x); mix gently and spin down briefly.

a.Add Mix A to Mix B, vortex for 5-15 sec and spin down briefly.
b.Incubate 30 min at Room Temperature (protect from direct light)
c. Drop-wise apply Mix A + Mix B solution to each well and mix by gently swirling the plate.
d.Incubate @ 37°C (5% CO_2) for 90 min - 3 hours (incubation period depends on the cell line).
e.Aspirate the medium from each well and add 2 ml of Complete Medium.
f. Stop Transfection after 24 - 48 hours.

Transfection Mix for different cell culture formats

Cell Culture vessel	DNA (ug) diluted in 10ul of serum-free medium	Volume (ul) of StoS transfection KIT 10x for the indicated ratio DNA/StS Transfection Kit 10x			Volume (ul) of Serum-free Medium for StS Transfection Kit 10x dilution		
		1:3	1:4	1:5	1:3	1:4	1:5
96-well plate	0.125	0.375	0.5	0.625	3.375	4.5	5.625
48-well plate	0.25	0.750	1.0	1.25	6.75	9.0	11.25
24-well plate	0.5	1.5	2.0	2.5	14.5	18.0	22.5
12-well plate	1.0	3.0	4.0	5.0	27.0	36.0	25.0
6-well plate	2.0	6.0	8.0	10.0	54.0	72.0	90.0

Comments and Suggestions

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- If **cytotoxicity** is observed:
  - _ remove the Transfection Mix after 1 hour
  - _ decrease the amount of plasmid DNA in the assay while keeping constant the DNA/StS Transfection Kit 10x ratio
  - _ check for toxicity of the expressed protein
  - _ verify that your plasmid DNA preparation is endotoxins-free

- If **low transfection efficiency** is observed:
  - _ increase the constant DNA/StS Transfection Kit 10x ratio
  - _ verify the quality of your plasmid DNA
  - _ try with Reverse Transfection
  - _remove cell culture medium just prior to apply Transfection Mix, drop-wise apply Transfection Mix in a volume corresponding to 1/4 of growing medium (e.g. 0.5ml for a 6-well plate), evenly distribute by gentle swirling, incubate for 2h @ 37°C while swirling the plate every 20 min, finally remove Transfection Mix and add the apporpriate volume of Complete Medium (e.g. 2ml for 6-well plate).