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For Research Purposes Only

APE-BridgePath Scientific
4841 International Blvd.
Suite 105
Frederick, MD 21703

Phone: 240-436-6146
Fax: 240-436-6152

www.apebridgepath.com

GETITIN™

Transfection Reagent

Introduction

APE-BridgePath Scientific's GETITIN™ Transfection Reagent is an easy to use a proprietary formula of polyethylenimine derivatives that is compatible with serum and other cell culture additives.

Requirements:

- Store at 4 °C. Warm to 20-50 °C before use if precipitation is seen
- Adherent Cells: healthy, recently plated (1-2 days prior) in the desired growth chambers (see below) and grown to 20-50% confluence.
- Suspension Cells: healthy cells in fresh medium at ~0.2-0.4 x 10⁶ cells/ml (mammalian) or ~2-4x10⁶ cells/ml (Schneider S2 cells).
- DNA: CsCl pure or equivalent; of O.D. 260/280 ~ 1.7-1.85; re-suspended at 1 mg/ml in TE or water
- Cell Culture Medium: pre-warmed to 20 °C.

Summary of transfection conditions (example for 24 well plate):

For transfection of 0.5 µg of DNA:

1. Suspension A: 0.5 µg of DNA is diluted to 20 µl in medium (20 °C)
2. Suspension B: 1.3 µl of GETITIN™ is diluted to 20 µl with medium (20 °C)
3. Mix Suspension B with Suspension A (not A with B!)
4. Incubate 10-15 min at 20 °C
5. Add to cells in 415 µl of medium (20 °C)
6. Mix the GETITIN/DNA complex with the cells and incubate for 24-48 h

Protocol:

1. Warm GETITIN™ solution to 20 °C. If a precipitate is present, warm the solution to 50 °C to resuspend the precipitate, then cool to 20 °C.
2. Warm medium to at least 20 °C.
3. Use the table below to decide on the amount of DNA and GETITIN™ required
4. Take two sterile tubes of sufficient volume to contain volume of Suspensions.
5. Label the tubes (from 4) A and B
6. Add the volume of medium required (as described in the table) to each tube A and B (from 5).
7. Add the required amount of DNA (as described in the table) to tube A (from 6).
8. Mix the contents of tube A (from 7)
9. Add the required amount of GETITIN™ (as described in the table) to tube B (from 6).
10. Mix the contents of tube B (from 9).
11. Transfer the contents of tube B (from 10) into tube A (from 8)
12. Discard empty tube B (from 11)
13. Mix the contents of tube A (from 11). You may vortex for 10 s.
14. Incubate tube A (from 13) 10-15 min at 20 °C
15. For adherent cells: Remove spent medium from culture.
16. For adherent cells: Add the required amount of pre-warmed medium (as described in the table) to the culture chamber. For suspension cells, plate cells such that the volume of culture plated in the chamber is equivalent to the ml of medium required above the culture (as described in the table).
17. Add the contents of tube A (from 14) to the culture.
18. Discard empty tube A (from 17)
19. Incubate the culture for 24-48 h under growth conditions
20. Measure expression, or change medium and incubate culture for another 24-48 h prior to assessment.

Optimized DNA/GETITIN quantities for various cell culture environments:

Culture Environment	Area cm²	µg of DNA	µl of GETITIN™	ml of Suspensions	ml of prewarmed medium above culture
96 well plate	0.32	0.084	0.22	0.0033	0.07
48 well plate	0.95	0.25	0.65	0.01	0.208
24 well plate	1.9	0.5	1.3	0.02	0.415
12 well plate	3.8	1	2.6	0.04	0.83
35 mm dish	8	2.1	5.5	0.085	1.75
6 well plate	9.5	2.5	6.5	0.1	2.1
60 mm dish	21	5.5	14.4	0.221	4.6
25 cm ² flask	25	6.6	17	0.263	5.5
100 mm dish	55	14.5	38	0.58	12
75 cm ² flask	75	20	51	0.79	16.4
150 mm dish	148	39	101	1.56	32
150 cm ² flask	150	40	103	1.58	33
175 cm ² flask	175	46	120	1.84	38
225 cm ² flask	225	59	154	2.37	49
850 cm ² bottle	850	223	582	8.95	186

Qualified for transfection in:

NIH-3T3	CV-1	MCF-7
CaCo2	HEK-293	MRC-5
CHO	VERO	Primary endothelial cells
COS-1	HeLa	Primary hepatocytes
COS-7	Jurkat	Primary human fibroblasts