FuGENE® HD

F200 (200µL) and F500 (500µL)

OVERVIEW

FuGENE® HD Transfection Reagent is a multi-component reagent that forms a complex with DNA, then transports the complex into animal or insect cells. This reagent is suitable for use in media with or without serum, and for transient or stable transfection. With FuGENE HD, there is no need to change media after an incubation with the transfection mix because of its minimal cytotoxicity.

STORAGE & HANDLING

- Store FuGENE HD at +4C with the lid tightly closed in its original glass vial. If the reagent is stored below 0C temporarily, briefly warm it to 37C to dissolve any precipitate.
- Always bring FuGENE HD to room temperature and mix well prior to use.
- When pipetting FuGENE HD from its glass vial into any working reagent mixes, DO NOT allow the reagent to contact the plastic walls of the tube. Do not use siliconized pipette tips or tubes.

OPTIMIZING TRANSFECTION CONDITIONS

- Use the LightSwitch™ Transfection Optimization Kit (Cat# TFXOPT).
- Use of low-passage cell lines is strongly recommended as highly-passaged cells generally yield inconsistent transfections.
- Test a variety of uL FuGENE HD to ug DNA ratios ranging from 2:1 to 6:1.
- Other parameters to vary include the amount of plasmid DNA used, seeded cell density, and duration of transfection.

RELATED PRODUCTS

Item	Catalog no.
LightSwitch™ Transfection Optimization Kit	TFXOPT
LightSwitch™ Assay Reagent™ (100 assays)	LS010
LightSwitch™ Assay Reagent™ (1,000 assays)	LS100
GoClones™ Promoters	S7- - - - -
GoClones™ Promoter Controls	S79- - - -
GoClones™ 3´UTRs	S8- - - - -
GoClones™ 3´UTR Controls	S89- - - -

GENERAL TRANSFECTION WORKFLOW

NOTE: Details related to optimal seeding density, duration of transfection, and amounts of plasmid or FuGENE HD to add will vary widely by cell line, conditions, and assay type. For specific GoClone transfection protocol examples, see our online resources page at http://switchgeargenomics.com/resources/.

DAY 1: Seed cells so they will be 80%-90% confluent at the time of transfection. For 96-well format, seed cells in 100uL total volume per well.

DAY 2:

1) Pre-warm serum free media (like Invitrogen's Opti-MEM) to 37C and bring FuGENE HD to room temperature (mix well).
2) Calculate the amount of DNA, FuGENE HD, and Opti-MEM that will be added to each transfection well. For 96-well format, the total volume added to each well should be 5µL. We suggest starting with 50–100ng of DNA per well and testing 3:1 and 6:1 ratios of FuGENE HD to DNA (µL:ug). To prepare transfection complexes for other well formats, proportionally change the quantity of all components based on the relative difference in surface area of the well size being used.
3) Mix plasmid DNA and Opti-MEM.
4) Add FuGENE HD to DNA + Opti-MEM mix without touching the sides of the tube. Mix well.
5) Incubate the transfection complex for 30 minutes at room temperature.
6) Add the transfection complex to the cells by gently dripping the transfection mix into the well.
7) Incubate the cells for 18–72 hours before assessing the results. Transfection times will vary by cell type and assay type.

Quality Control

Function is assessed using a reporter gene assay in COS-7 cells. Activity must be at least 75% compared to a reference lot. Cell growth is assessed using a cell proliferation test which is an indirect measure of cytotoxicity. Cells are also tested for overall viability. A detailed Certificate of Analysis is available upon request.

Label License

You shall use this Product in accordance with all applicable laws, rules, and regulations. You may not attempt to reverse engineer this Product. If you are not willing to accept these conditions, the distributor from whom the Product was purchased will accept the return of the unopened Product and provide you with a full refund. However, in the event this Product is opened, you agree to be bound by the conditions of this limited use statement.