HEK293-6E Cell Preparation for Transfection (20101121JV)

Purpose: The purpose of the guidelines below is to provide a procedure to generate HEK293-6E cells that allow optimal transient protein expression.

1. Materials, Conditions:
Culture media: FreeStyle™ 293 Expression Medium (Invitrogen Cat. 12338-018)
Incubator: 8% CO2, 37^{o}C, humidified atmosphere. Orbital shaking 90 rpm for 1L culture, 130 rpm for smaller volumes.
Labware: Ploycarbonate shake flasks with vented lids. The flasks can be re-used after washing and autoclaving, but have to be checked carefully for potential cracks to avoid media spills in the incubator. The following sizes are used for specific volumes:

Flask Volume (ml)	Culture Volume (ml)	rpm	Corning Cat.#
2800	1000	90	431252
1000	400	130	431147
250	100	130	431144
125	30	130	431143

2. Cell Numbers
The following cell numbers guarantee optimal protein expression

A. Grow cells at least until they are 2x10E6 but no denser than 3x10E6.
B. When splitting the cells, dilute them to no less than 1x10E6.
C. Cells need to double with a 24 hr doubling rate to be ready for transfection
D. Ideally have the cells double two consecutive times at maximum doubling rate ("log phase") before Transfection
E. At transfections dilute cells to 1x10E6.