DNA transfection of *Drosophila* S2 cells using "Biontex K2® Transfection System"

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Materials and Methods

Cell culture

The S2 cell line was derived from a primary culture of late stage (20-24 hours old) *Drosophila melanogaster* embryos (Schneider, 1972). Many features of the S2 cell line suggest that it is derived from a macrophage-like lineage. S2 cells grow at room temperature without CO_2 as a loose, semi-adherent monolayer in tissue culture flasks and in suspension in spinners and shake flasks. The complete medium for S2 cells is *Schneider's Drosophila Medium* containing 10% heat-inactivated FBS. This medium is used for transfection. S2 cells reached a confluence when cell density is between 6 to 20 x 10^6 cells/ml. Transfection was performed when cells had reached a confluency of 90-100%. The day before transfection 2 x 10^5 cells were plated in a 48-well dish (Becton Dickinson Labware Europe) in 250 µl of complete growth medium and incubated at 28 °C.

Cell transfection

Cells were treated with K2® Multiplier, 2 hours before DNA transfection. Each well of rows C and D of the plate contain 2,5 µl of K2® Multiplier and rows E and F contain 5 µl. 20 µg of a plasmid-DNA (pIZT/V5-His Invitrogen) encoding a protein named *Ae*-ENO (45 kDa) produced by *Aphidius ervi* (Haliday) (Hymenoptera, Braconidae) was added to 1000 µl of serum-free *Schneider's Drosophila Medium* (Solution A) and mixed. As indicated by the manufacturer were prepared four solutions (Solutions B):

B(1:2): adding 9µl of K2® Transfection Reagent to 225 µl of serum free medium

B(1:3): adding 13,5µl of K2® Transfection Reagent to 225 µl of serum free medium

B(1:4): adding 18 µl of K2® Transfection Reagent to 225 µl of serum free medium

B(1:5): adding 22,5µl of K2® Transfection Reagent to 225 µl of serum free medium

225 µl of Solution A was added to each Solution B, mixed and incubated at room temperature for 15 min.

The DNA-lipid complex was added to cells after incubation as indicated by the manufacturer. The 48-well plate must contain the following combinations (Figure1):

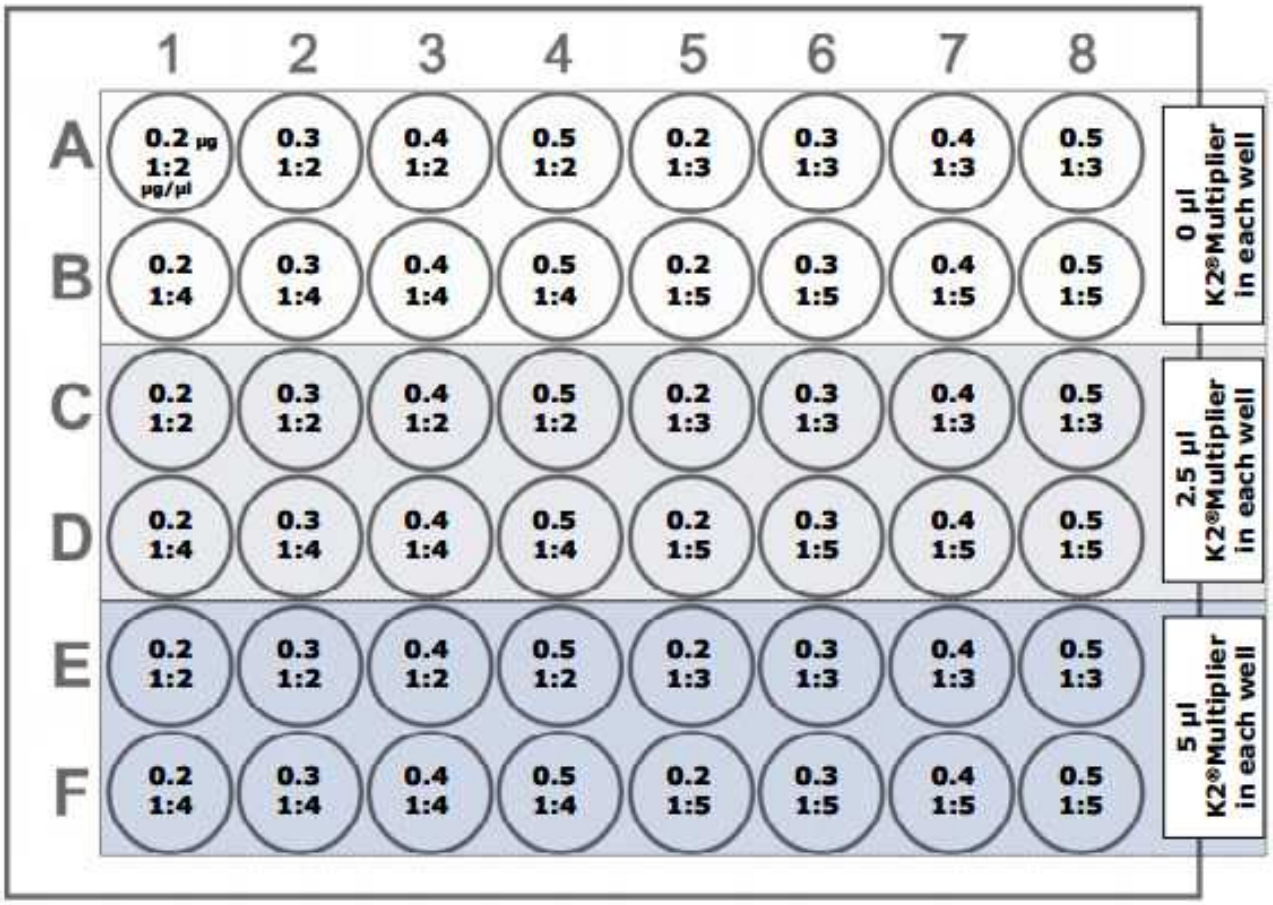

Figure 1. 48-well plate.Each well of the plate contains a particular combination of DNA/ K2® Transfection Reagent and K2® Multiplier.

Transfections were incubated at 28 °C and, after 24 h the transfection mixture was removed (centrifuging at 1000 *g* for 3 min at room temperature) and replaced with fresh complete medium. Transfection results were estimated by fluorescence microscopy (to see expression of the GFP encoded by the plasmid) and western blotting.

Results

24 h after transfection, cells were observed with a fluorescence microscope to evaluate the expression of GFP encoded by the plasmid.

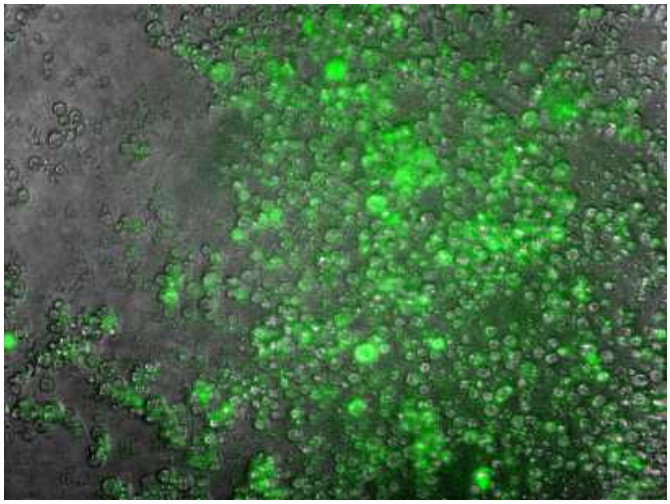

Figure 2. GFP activity

For western blot analysis we loaded different amounts of proteins derived from the wells chosen at random. There are no significant differences in the results of the transfection. Fig 3 shows the results of western blot. In lane 1, 2, 3, 4, 5, 6 and 7 we loaded proteins taken 48 h after transfection derived from A1, A6, C1, C8, D1, E3 and F3. In lane 8 and 9 we loaded proteins taken 72 h after transfection derived from well B8 and D5 72 h after transfection.

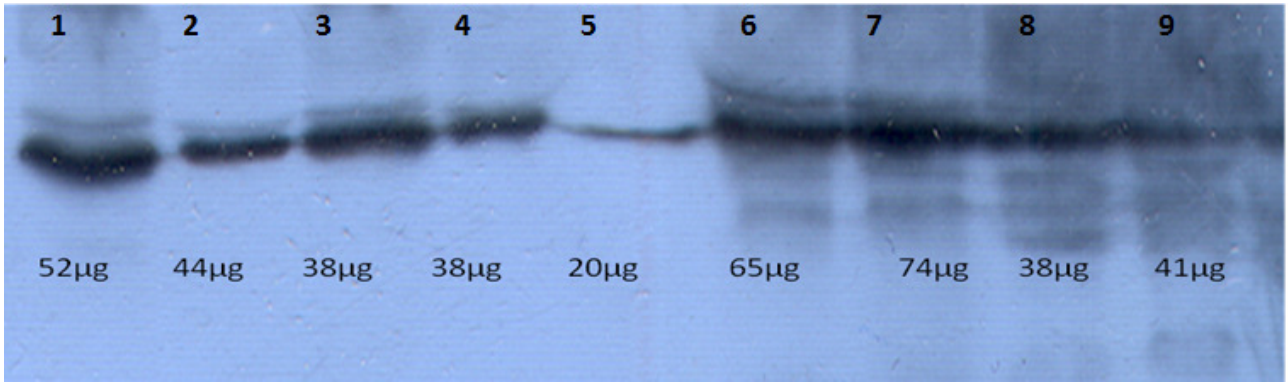

Figure 3. Western blot. It is indicated the amount of protein loaded.

Conclusions

For the western blot DNA encoding *Ae*-ENO was transfected into S2 cells using the K2® Transfection system. The western blot shows a band of the expected size (~45 kDa). Fluorescence microscopy shows also successful transfection.