



Epizone: Interlaboratory Ring Trial to Compare Dna Transfection Efficiencies

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EPIZONE: Interlaboratory Ring Trial to compare DNA transfection efficiencies.

Chemical-based transfection of DNA into cultured cells is routinely used to study for example viral or cellular gene functions involved in virus replication, to analyse cellular defence mechanisms or develop specific strategies to interfere with virus replication. Other applications include rescue of viruses by reverse genetics and/or generation of mutated viruses. A large number of transfection chemicals like calcium phosphate, branched organic compounds, liposomes, cationic polymers etc. are available on the market which are used by different laboratories for different cell lines. To obtain an overview on the efficiencies of varying transfection procedures, an interlaboratory ring trial was initiated within EPIZONE theme 5. A total of 15 participating laboratories from 7 member institutions received RK13 cells, plasmid DNA encoding firefly luciferase under the transcriptional control of the human cytomegalovirus major immediate early promoter, a specially developed lysis buffer and a detailed protocol. Transfected cells were harvested in the laboratories of the participants, frozen and sent to the FLI where both the luciferase activity and protein content of the individual samples were determined to compare transfection efficiency between laboratories with the same protocol and equipment. In addition some laboratories sent samples from cells they are routinely using, transfected with the provided firefly luciferase plasmid, to allow comparison of transfection efficiency between different cell types. About 50 different samples were analysed and the luciferase activity per nanogram total protein (RLU/ng) was determined. The results revealed for RK13 cells a large range of specific luciferase activities between laboratories and, in comparison to RK13 cells, also varying transfection efficiencies for other the cell lines. Details will be presented.

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