

P005 Inhibition of reporter gene expression by co-transfection of the cytomegalovirus promoter/enhancer

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Co-transfection of a plasmid expressing a reporter gene together with a plasmid encoding a regulatory RNA or protein is commonly used to study factors that control gene expression at the transcriptional or translational level. We have previously reported that co-expression of the Epstein-Barr virus-encoded RNA, EBER-1, stimulates the expression of various reporter genes in several cell types. The mechanism of action of EBER-1 is believed to involve inhibition of activation of the eIF2 α kinase, PKR. However we found previously that EBER-1 is apparently able to exert a stimulatory effect even in PKR $^{-/-}$ cells, suggesting a novel mode of action. We now show that this result is probably an artefact, arising from an inhibitory effect on reporter gene expression of the control pcDNA3 plasmid employed. The inhibitory effect of pcDNA3 is due to the strong cytomegalovirus (CMV) enhancer/promoter element present in this vector and is not seen when pcDNA2, which lacks the CMV sequence, is used. Further studies are required to determine whether the CMV sequence inhibits reporter gene expression at the transcriptional or translational level. The ability of EBER-1 to stimulate reporter gene expression, as well as the role played by PKR in such effects, needs to be reinvestigated in the light of these results.