Recombinant expression of the rabies virus glycoprotein in eukaryotic cell system.

A. Giordano Mendoza[1]; A. Torres López[1]; A. Schichera[1]; D. Garanzini[2]; G. Calamante[1]; M.P. Del Médico Zajac[1].

[1]Instituto de Biotecnología (IABiMo), UEDD INTA-CONICET. [2]Dpto. de Ciencias Aplicadas y Tecnología, Universidad Nacional de Moreno.

INTRODUCTION

ELISA for determining rabies antibodies are routinely used to evaluate immune response induced for new vaccine candidates. Our research group has developed an in-house ELISA using inactivated and purified RABV as coating antigen. However, we observed considerable variability in antigen purification yields within different inactivated RABV batches.

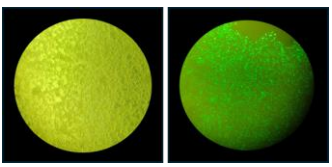
In this study, **we evaluate expressing the RABV glycoprotein in eukaryotic cells.** In the future, this recombinant protein could be used as a coating antigen for an ELISA that replace imports and circumvent the need for large-scale rabies virus production.

METHODS

1. Amplification of rabies glycoprotein ectodomain (RGe) sequence by PCR
2. Cloning RGe sequence in pSecTag2 vector
3. Transfection of HEK293T cells
4. Evaluation of RGe expression by WB in cell pellet (P) and supernatant (SN) at 48 and 72 h post transfection.
5. Extraction of RGe from cell pellet using Native (N) or Denaturing (D) buffers.

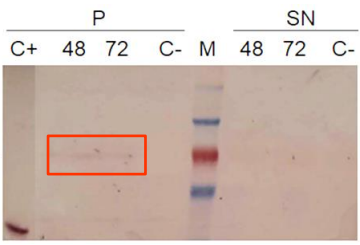
RESULTS

Transfection of HEK293T cells

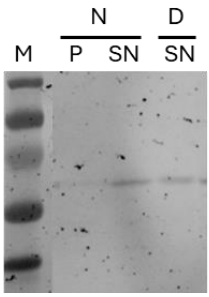


pST-RGe pCDNA-GFP

Detection of RGe in cell pellet



Extraction of RGe in native conditions



CONCLUSION

➽ The ectodomain of the RABV glycoprotein is transiently expressed in HEK293T cells and remains retained within the cells.

➽ The recombinant protein can be extracted from the intracellular fraction under native conditions.

delmedicozajac.maria@inta.gob.ar