

WUHAN INSTITUTE OF VIROLOGY BUDGET JUSTIFICATION, SUBAWARD

A. Senior/Key Personnel:

Co-Investigator, Dr. Zhengli Shi, a Senior Research Scientist at the Chinese Academy of Science's Wuhan Institute of Virology will commit (b) (4), (b) (6) per year (b) (4), (b) (6) to this project to refine study protocols, coordinate research, oversee implementation of all activities, analyze data, lead regular meetings with other PD/PI and Other Senior/Key Personnel as well as draft papers. Dr. Xingyi Ge, Senior Research Technician, will commit (b) (4), (b) (6) per year to perform all laboratory work and directly supervise the laboratory technician.

B. Other Personnel

One laboratory technician will commit (b) (4), (b) (6) per year (b) (4), (b) (6) each to this project to perform all required laboratory assays and maintenance as well as participate in selected meetings, perform research for papers, and assist Dr. Shi in performing the work under this award.

All Wuhan Institute of Virology salaries include the US "overhead" or "fringe", so this is not calculated separately.

C. Equipment

No equipment over \$5,000 will be purchased.

D. Travel

We are requesting \$2,060 per year for all years for Senior/Key Personnel Dr. Shi and Dr. Ge to travel to Shanghai to visit partner laboratory at East China Normal University (ECNU) and meet with the PD/PI as well as with collaborators on this proposal: these include EcoHealth Alliance, East China Normal University, Yunnan CDC, Shanghai CDC, and Guangdong CDC. Travel is calculated at one round trip airfare from Wuhan to Shanghai (\$300), three-night hotel in Shanghai (\$150 per night), and four days per diem (at \$70 per day)

F. Other Direct Costs

We are requesting support for laboratory experiments and related costs.

RNA Extractions

We will be running RNA Extractions for 1,000 bats per year (three samples per bat: oral, anal, and blood) in each year of the project. This will cost \$13,922 per year (QIAamp ViralRNA Mini Kit with Axygen Pipette Tips and Filter Tubes at \$4.64 per sample). Extracted RNA per animal will be pooled.

RT-PCR

Costs for 1-Step RT-PCR assays for Coronavirus conducted on 1,000 samples per year for each year of the project total \$7,123 and are detailed as follows: Superscript III one step kit (\$5.18 per sample); Platinum Tag DNA Polymerase (\$0.57 per sample); nuclease-free water (\$0.16 per sample); and Axygen Pipette Tips and Filter Tubes (\$1.21 per sample).

DNA Sequencing

In each year of the project, DNA Sequencing will be performed on 3,200 samples at a cost of \$2.91 per reaction. We request a total of \$9,325 per year in each year.

Laboratory Supplies

We request support for *in vitro* infection experiments using pseudoviruses carrying the spike proteins (wild type or mutants) or live viruses in cell lines of different origins, binding affinity assays between the spike proteins (wild type or mutants) and different cellular receptor molecules, and humanized mouse experiments.

In Year 1, \$65,367 is requested: Lipofectamine2000 transfection reagent at a cost of \$2,428; cell lines from bats and other mammals including primates and humans at a cost of \$971; *in vitro* infection experiments require GIBCO Fetal Bovine Serum (\$3,562), GIBCO antibiotic antimycotic (\$563), GIBCO medium (\$2,914) as well as \$19,426 for Corning Cell culture; receptor-mutant pseudovirus binding assays require Luciferase assay system E1500 (\$858), pseudovirus package (\$3,885), and sequencing (\$22,664); \$8,094 is required for protein expression from the binding affinity assays.

In Year 2, \$70,385 is requested: Lipofectamine2000 transfection reagent at a cost of \$2,428; cell lines from bats and other mammals including primates and humans at a cost of \$971 – sufficient cell lines will be established by the end of Year 2, so this cost requirement will discontinue in Years 3-5; increased number of *in vitro* infection experiments require slightly more funding for GIBCO Fetal Bovine Serum (\$4,047) as well as GIBCO antibiotic antimycotic (\$563), GIBCO medium (\$2,914) as well as \$19,426 for Corning Cell culture; receptor-mutant pseudovirus binding assays require Luciferase assay system E1500 (\$858), pseudovirus package requirements will approximately double from Y1 (\$6,799), and sequencing (\$22,664); \$9,713 is required for protein expression from the increased Year 2 number of binding affinity assays at a slightly higher cost than year one as well.

In Years 3, 4 and 5, \$89,002 is requested per year: Lipofectamine2000 transfection reagent at a cost of \$2,428 per year; increased number of *in vitro* infection experiments require slightly more funding for GIBCO Fetal Bovine Serum (\$5,828 per year) as well as GIBCO antibiotic antimycotic (\$563 per year), GIBCO medium (\$2,914 per year) as well as \$19,426 per year for Corning Cell culture; receptor-mutant pseudovirus binding assays require Luciferase assay system E1500 (\$858 per year), pseudovirus package requirements will be \$6,799 per year, sequencing (\$22,664 per year) and gene synthesis (\$12,915 per year) will also be required; \$9,713 per year is required for protein expression from binding affinity assays; in only years 3, 4, and 5 humanized mouse *in vivo* experimental animals will be raised at an annual cost of \$4,857 per year.

H. Indirect Costs

We are requesting an extremely indirect cost of 8% on all direct costs.