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Generation And Biophysical Characterization of New A-nsp13 Helicase Mabs, as a Powerful Tool for Studying Sars-cov-2 Replication and Therapeutic Targeting

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Nsp13 helicase of SARS-CoV-2 plays a crucial role as part of the replication complex (alongside nsp7, nsp8, nsp12 proteins), ensuring efficient viral RNA synthesis, replication and transcription. Due to its function and high sequence conservation, Nsp13 is a promising therapeutic target for coronaviridae infections. To validate the MoA of inhibitors or degraders toward helicase in infected cells, specific mAbs for immunoassays are missing. We reported the generation, the biophysical characterization and validation of new anti-NSP13 mAbs, raised in rabbits using full-length protein as immunogen. Positives were identified by B-cells cloning and ELISA screening then produced as recombinant mAbs. Antibodies homogeneity (LC-SEC), affinity and epitope binning (BLI) were established. Detection of overexpressed Nsp13 in A549 cells was checked both by WB, IP and ad hoc developed MSD immunoassay. From rabbit immunization 5 α-NSP13 mAbs were identified. All showed high affinity and specificity, and stable complex-halflife (kd < 10-5 s-1). They proved suitable for WB analysis, while requiring optimization for IP use. They were coupled based on epitope and immunoassay developed using Meso Scale Discovery (MSD) technology. In conclusion, new anti-NSP13 mAbs were generated, characterized and applied to set-up a sensitive MSD assay for helicase binders orthogonal characterization.