Cymbidium mosaic virus (CymMV) is the most prevalent virus infecting orchids worldwide. Reliable detection method is required to investigate for virus-free orchid propagation materials. This study aimed to produce monoclonal antibodies (MAbs) against CymMV and develop the detection protocol in ELISA format. Four hybridoma cell lines producing anti-CymMV antibodies were obtained from fusions namely Cy1, Cy2, Cy3 and Cy4. All of them were highly specific to CymMV and no cross reaction with other plant viruses or healthy sap was observed. Comparison of direct, indirect and sandwich enzyme-linked immunosorbent assay (ELISA) for the detection of CymMV demonstrated that indirect PTA-ELISA gave highest sensitivity which as low as 0.25 ng virus could be detected. Thirty young leaf and root samples from Dendrobium spp. and tissue culture were used in the experiment to compare the efficiency of our developed ELISA with commercially available GLIFT kit. All of the samples gave the same results in which 76.67% of the samples were positive by 3 methods. Interestingly the signals showed higher virus accumulation in root than leaf samples among 50% of the positive samples. This study confirmed the efficiency these serological detection by using high quality and specific MAbs for the detection of this virus in orchids.