SARS Coronavirus Nucleocapsid Protein
Monoclonal Antibodies

Antigen Used for Immunization
The nucleocapsid protein of SARS-CoV was separated into two parts (N1, 1-183 aa, and N2, 166-422 aa), produced in and purified from *Escherichia coli*.

Method of Immunization
BALB/c mice (8 weeks old) were subcutaneously immunized with 10, 20, or 30 μg of immunogen resuspended in PBS plus equal volumes of complete Freund’s adjuvant, followed by two booster immunizations at 2-week intervals. The titer of mouse serum was determined by ELISA.

Parental Cell Line Used for Fusion
Splenocytes from immunized mice were fused with SP2/0 myeloma cells.

Selection and Cloning Procedure
Cell culture supernatants from hybridoma colonies were screened by ELISA using the purified N1 or N2 as a coating antigen. Cells from positive wells were expanded and re-tested. Cultures that remained positive were subcloned to generate stable hybridoma cell lines by limited dilution methods.

Heavy and Light Chains of Immunoglobulin
Six MAbs were IgG1 class (N1.1D7, N1.1H2, N1.2C5, N1.2D3, N1.2F6, N2.1H6), two MAbs were IgG2b (N1.1B1 and N2.1C4), and N1.1A6 was IgG3.

Specificity
Specificity was evaluated by Western blot analysis.

Specific Antigen Identified
N1.1A6, N1.1B1, N1.1D7, N1.1H2, N1.2C5, N1.2D3, and N1.2F6 react with N1 (1-183 aa) while N2.1C4 and N2.1H6 react with N2 (166-422 aa).

Availability
<table>
<thead>
<tr>
<th>Tissue culture supernatant</th>
<th>Yes</th>
<th>No ✓</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascitic fluid</td>
<td>Yes ✓</td>
<td>No</td>
</tr>
<tr>
<td>Hybridoma cells</td>
<td>Yes ✓</td>
<td>No</td>
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</tbody>
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Reference

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