Fig. 1. (A) Validation of the immuno affinity purification procedure. To confirm the specificity of the IAP, we tested the binding of mouse hepatitis virus (MHV) mRNAs. These possess a 5'-cap structure, as was shown by Lai and co-workers (Lai et al., 1982a; Lai and Stohlman, 1981). Total cytoplasmic RNA extracted from MHV-infected cells was incubated with protein G sepharose beads, coupled with either the 7-methylguanosine (m7G)-cross reactive MAb H20 (αCap) or, as a negative control, the isotype-matched MAb R78 (αTcR). RNA, bound to the MAb-coupled beads, was eluted, separated in denaturing 1% agarose gels and hybridized to a radiolabeled oligonucleotide probe, complimentary to the 3’ end of the MHV genome. Untreated intracellular MHV RNAs served as a marker (M). The locations of MHV mRNAs 1 through 7 are indicated. MHV mRNAs exclusively bound to MAb H20-coupled beads.

(B) An 824-nt synthetic RNA was produced by transcription in vitro with bacteriophage T7 RNA polymerase using linearized plasmid DNA as a template. RNA synthesis was performed either in the absence or presence of the cap analogue M7G(5')ppp(5')G (Amersham). Equal amounts of capped (+) and non-capped (−) transcripts were either analyzed without further treatment by electrophoresis in denaturing formaldehyde-1% agarose gels (direct) or after IAP with beads coupled with MAb R78 (αTcR) or MAb H20 (αCap). Transcripts were visualized by hybridization with a specific radiolabeled oligonucleotide probe. As shown, the binding of synthetic RNA to MAb H20-coupled beads was specific and cap-dependent.