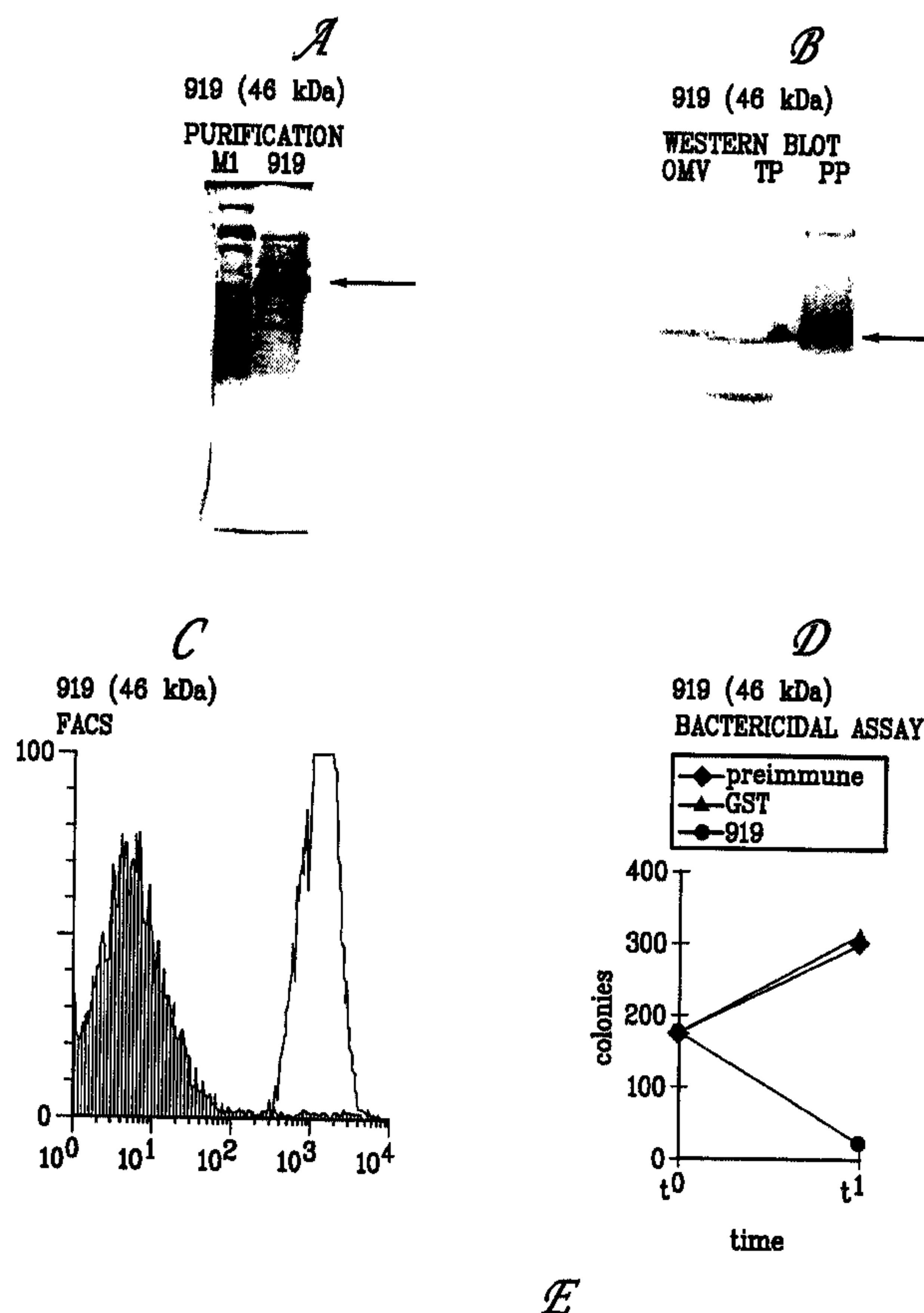




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(74) Agent: BORDEN LADNER GERVAIS LLP

(54) Titre : SEQUENCES GENOMIQUES DE NEISSERIA ET PROCEDES D'UTILISATION
(54) Title: NEISSERIA GENOMIC SEQUENCES AND METHODS OF THEIR USE



(57) **Abrégé/Abstract:**

The invention provides methods of obtaining immunogenic proteins from genomic sequences including Neisseria, including the amino acid sequences and the corresponding nucleotide sequences, as well as the genomic sequence of Neisseria meningitidis B. The proteins so obtained are useful antigens for vaccines, immunogenic compositions, and/or diagnostics.

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International Bureau

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US

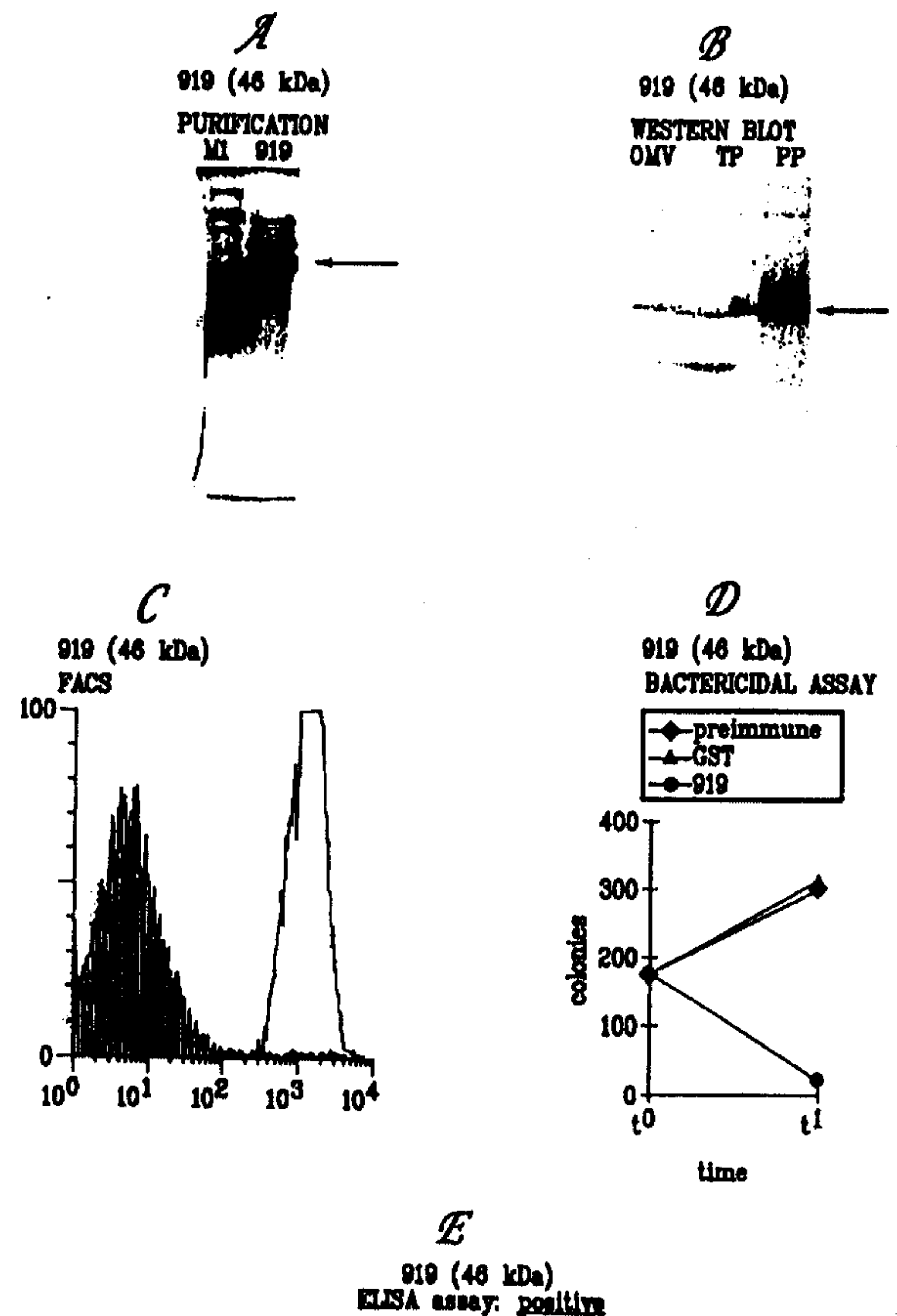
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(57) Abstract

The invention provides methods of obtaining immunogenic proteins from genomic sequences including *Neisseria*, including the amino acid sequences and the corresponding nucleotide sequences, as well as the genomic sequence of *Neisseria meningitidis B*. The proteins so obtained are useful antigens for vaccines, immunogenic compositions, and/or diagnostics.



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Claims

1. A method for identifying an amino acid sequence, comprising the step of searching for putative open reading frames or protein-coding sequences within one or more
5 of *N. meningitidis* nucleotide sequences SEQ ID NOs 1-961 and 1068, or even-numbered SEQ ID NOs from SEQ ID 962 to SEQ ID 1044.
2. A method according to claim 1, comprising the steps of searching a
10 *N. meningitidis* nucleotide sequence for an initiation codon and searching the upstream sequence for an in-frame termination codon.
3. A method for producing a protein, comprising the step of expressing a protein comprising an amino acid sequence identified according to any one of claims 1-2.
- 15 4. A method for identifying a protein in *N. meningitidis*, comprising the steps of producing a protein according to claim 3, producing an antibody which binds to the protein, and determining whether the antibody recognises a protein produced by *N. meningitidis*.
- 20 5. Nucleic acid comprising an open reading frame or protein-coding sequence identified by a method according to any one of claims 1-2.
6. A protein obtained by the method of claim 3.
- 25 7. Nucleic acid comprising one or more of the *N. meningitidis* nucleotide sequences SEQ ID NOs 1-961 and 1068, or even-numbered SEQ ID NOs from SEQ ID NO 962 to SEQ ID NO 1044.
- 30 8. Nucleic acid comprising a nucleotide sequence having greater than 50% sequence identity to a nucleotide sequence disclosed in the sequence listing SEQ ID NOs 1-961 and 1068, or even-numbered SEQ ID NOs from SEQ ID 962 to SEQ ID 1044.

- 123 -

9. Nucleic acid comprising a fragment of a nucleotide sequence disclosed in the sequence listing SEQ ID NOs 1-961 and 1068, or even-numbered SEQ ID NOs from SEQ ID 962 to SEQ ID 1044.

5 10. Nucleic acid according to claim 9, wherein the fragment is unique to the genome of *N. meningitidis*.

11. Nucleic acid complementary to the nucleic acid of any one of claims 7-10.

10 12. A protein comprising an amino acid sequence encoded within one or more of the *N. meningitidis* nucleotide sequences SEQ ID NOs 1-961 and 1068, or even-numbered SEQ ID NOs from SEQ ID 962 to SEQ ID 1044.

15 13. A protein comprising an amino acid sequences having greater than 50% sequence identity to an amino acid sequence encoded within one or more of the *N. meningitidis* nucleotide sequences SEQ ID NOs 1-961 and 1068, or even-numbered SEQ ID NOs from SEQ ID 962 to SEQ ID 1044.

20 14. A protein comprising a fragment of an amino acid sequence selected from the group consisting of one or more odd-numbered SEQ ID NOs 963-1037, amino acid sequences having greater than 50% identity with one or more odd-numbered SEQ ID NOs 963-1045, amino acid sequences encoded within one or more of the *N. meningitidis* nucleotide sequences SEQ ID NOs 1-961 and 1068, and amino acid sequences encoded by one or more even-numbered SEQ ID NOs from SEQ ID 962 to SEQ ID 1044.

25

15. Nucleic acid encoding a protein according to any one of claims 6-8.

30 16. A computer, a computer memory, a computer storage medium or a computer database containing the nucleotide sequence of a nucleic acid according to any one of claims 7-11.

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17. A computer, a computer memory, a computer storage medium or a computer database containing one or more of the *N. meningitidis* nucleotide sequences SEQ ID NOs 1-961.

5 18. A polyclonal or monoclonal antibody which binds to a protein according to any one of claims 12-14 or 6.

19. A nucleic acid probe comprising nucleic acid according to any one of claims 5, 7-10, or 15.

10

20. An amplification primer comprising nucleic acid according to any one of claims 5, 7-10, or 15.

15 21. A composition comprising (a) nucleic acid according to any one of claims 5, 7-10, or 15; (b) protein according to any one of claims 12-14; and/or (c) an antibody according to claim 18.

22. The use of a composition according to claim 21 as a medicament or as a diagnostic reagent.

20

23. The use of a composition according to claim 21 in the manufacture of (a) a medicament for treating or preventing infection due to Neisserial bacteria and/or (b) a diagnostic reagent for detecting the presence of Neisserial bacteria or of antibodies raised against Neisserial bacteria.

25

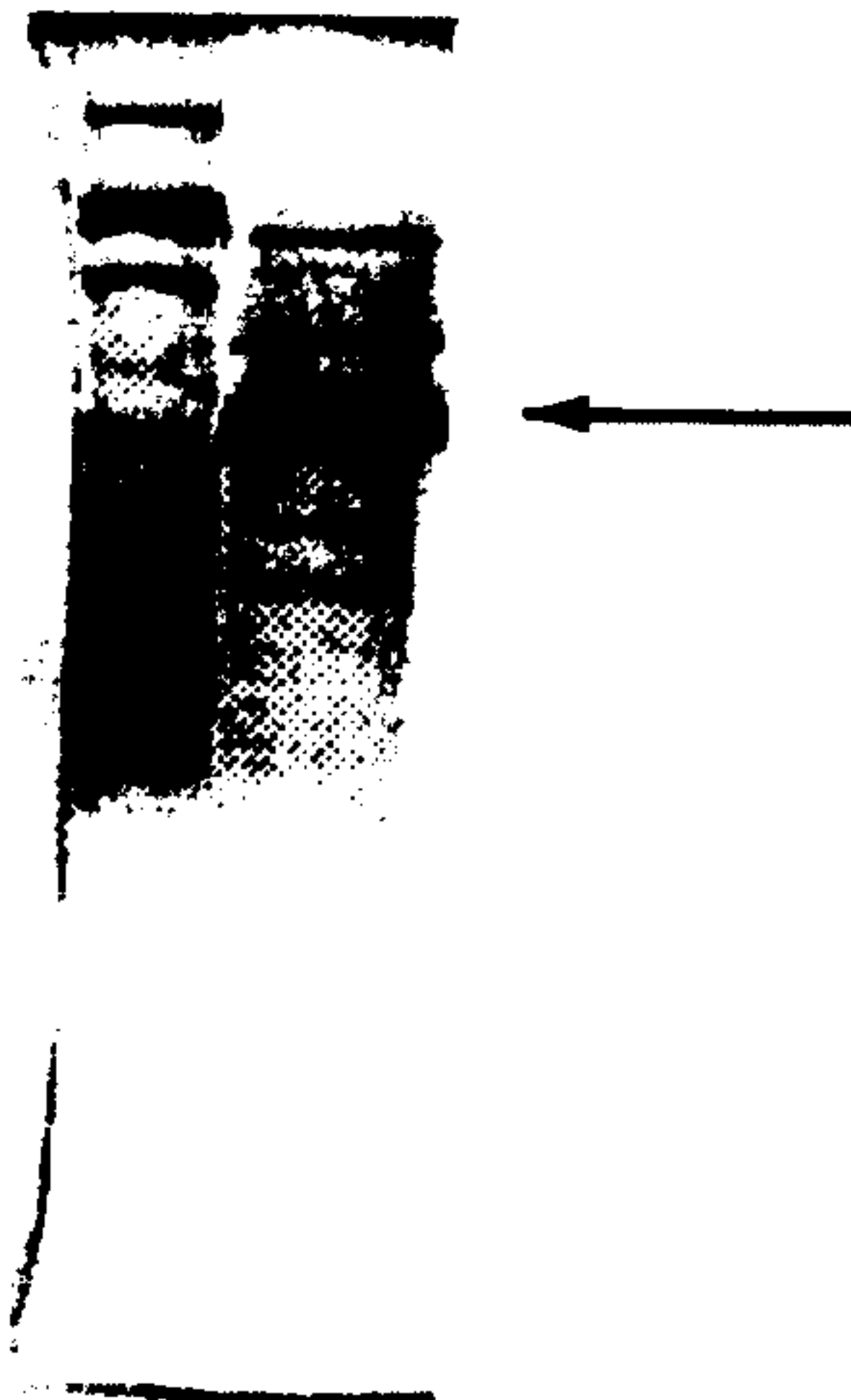
24. A method of treating a patient, comprising administering to the patient a therapeutically effective amount of a composition according to claim 21.

FIG. 1A

919 (46 kDa)

PURIFICATION

M1 919

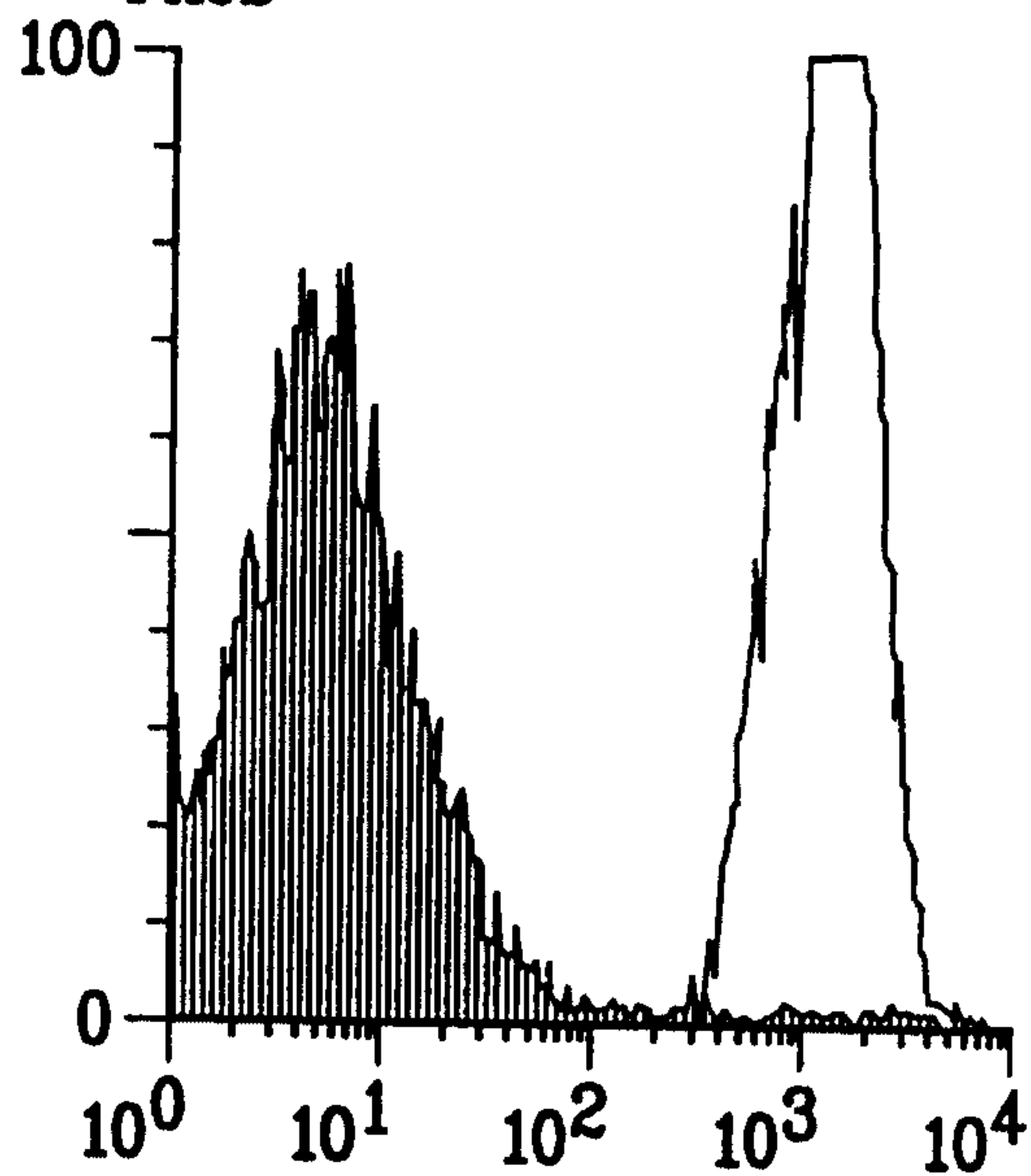
*FIG. 1B*

919 (46 kDa)

WESTERN BLOT
OMV TP PP*FIG. 1C*

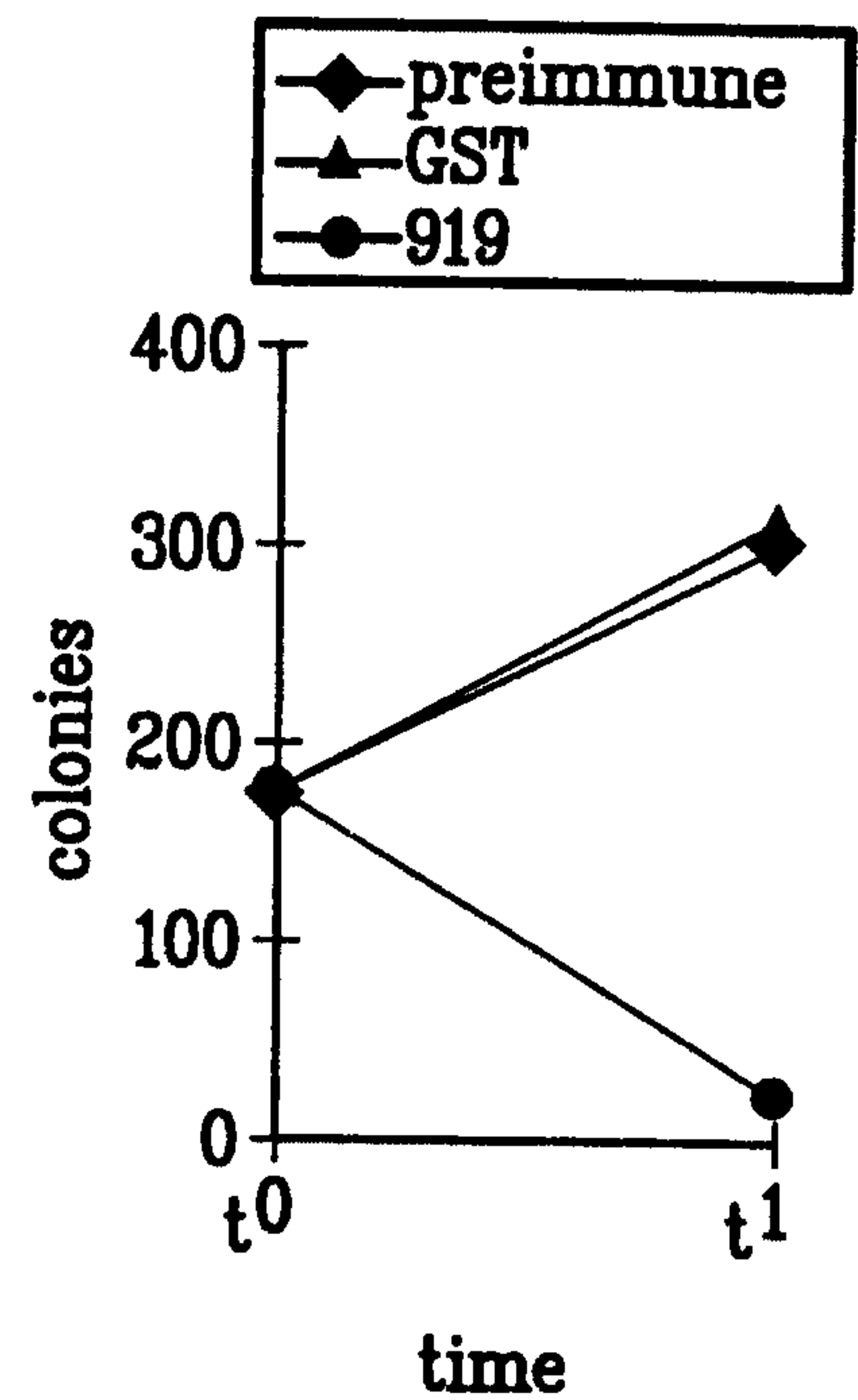
919 (46 kDa)

FACS

*FIG. 1D*

919 (46 kDa)

BACTERICIDAL ASSAY

*FIG. 1E*

919 (46 kDa)

ELISA assay: positive

FIG. 2A

279 (10.5 kDa)

PURIFICATION

M1 279

*FIG. 2B*

279 (10.5 kDa)

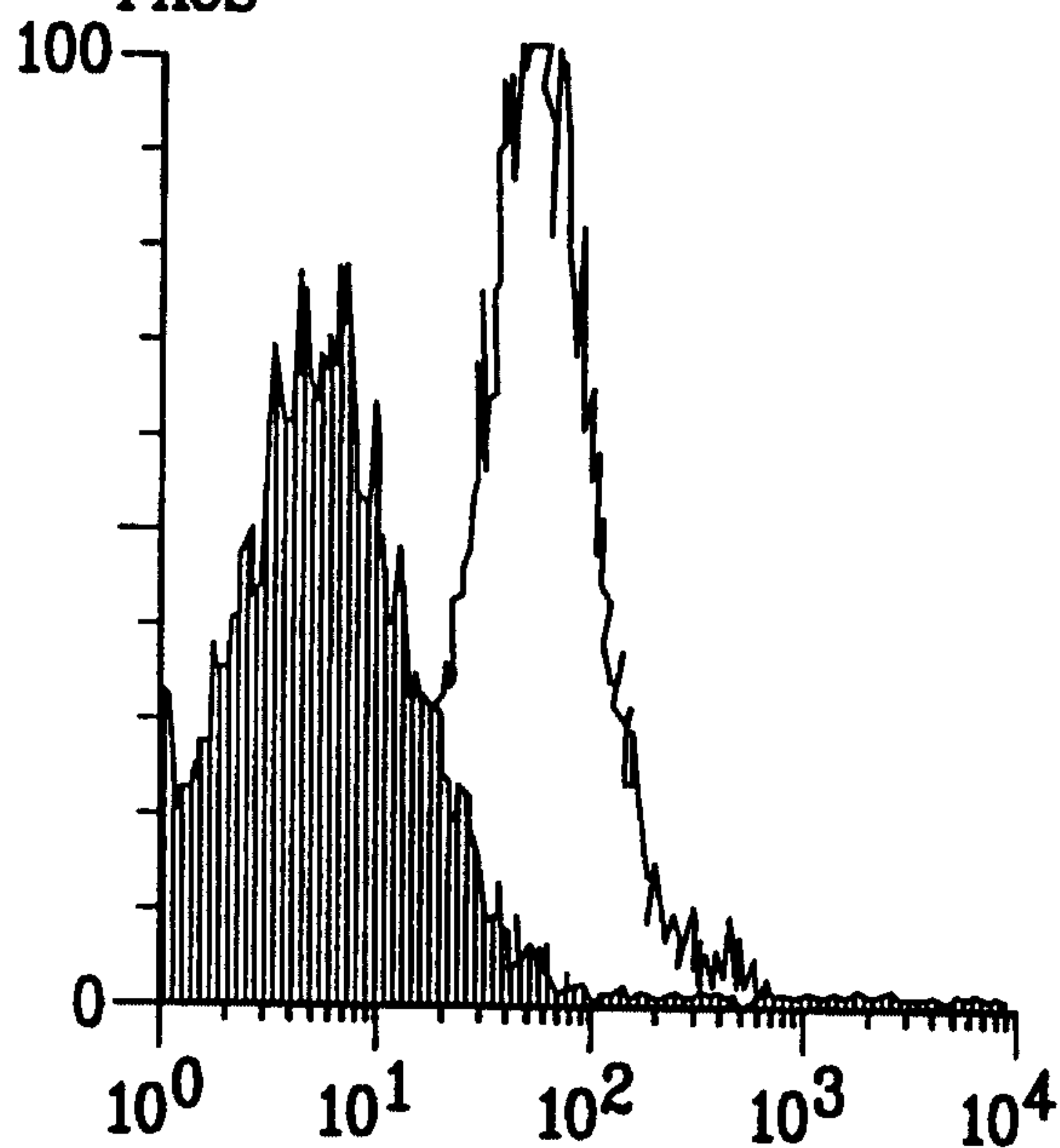
WESTERN BLOT

TP OMV

*FIG. 2C*

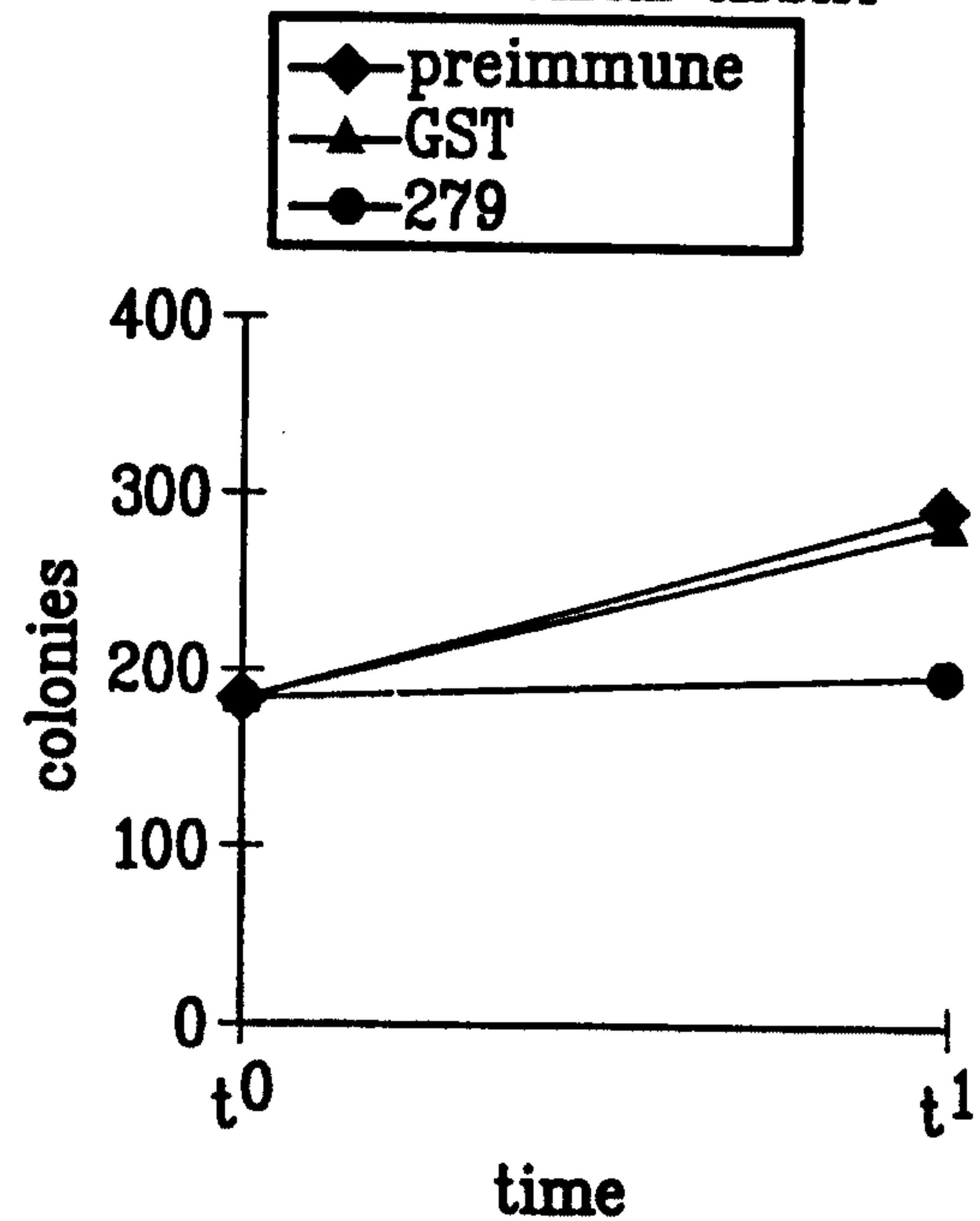
279 (10.5 kDa)

FACS

*FIG. 2D*

279 (10.5 kDa)

BACTERICIDAL ASSAY

*FIG. 2E*

279 (10.5 kDa)

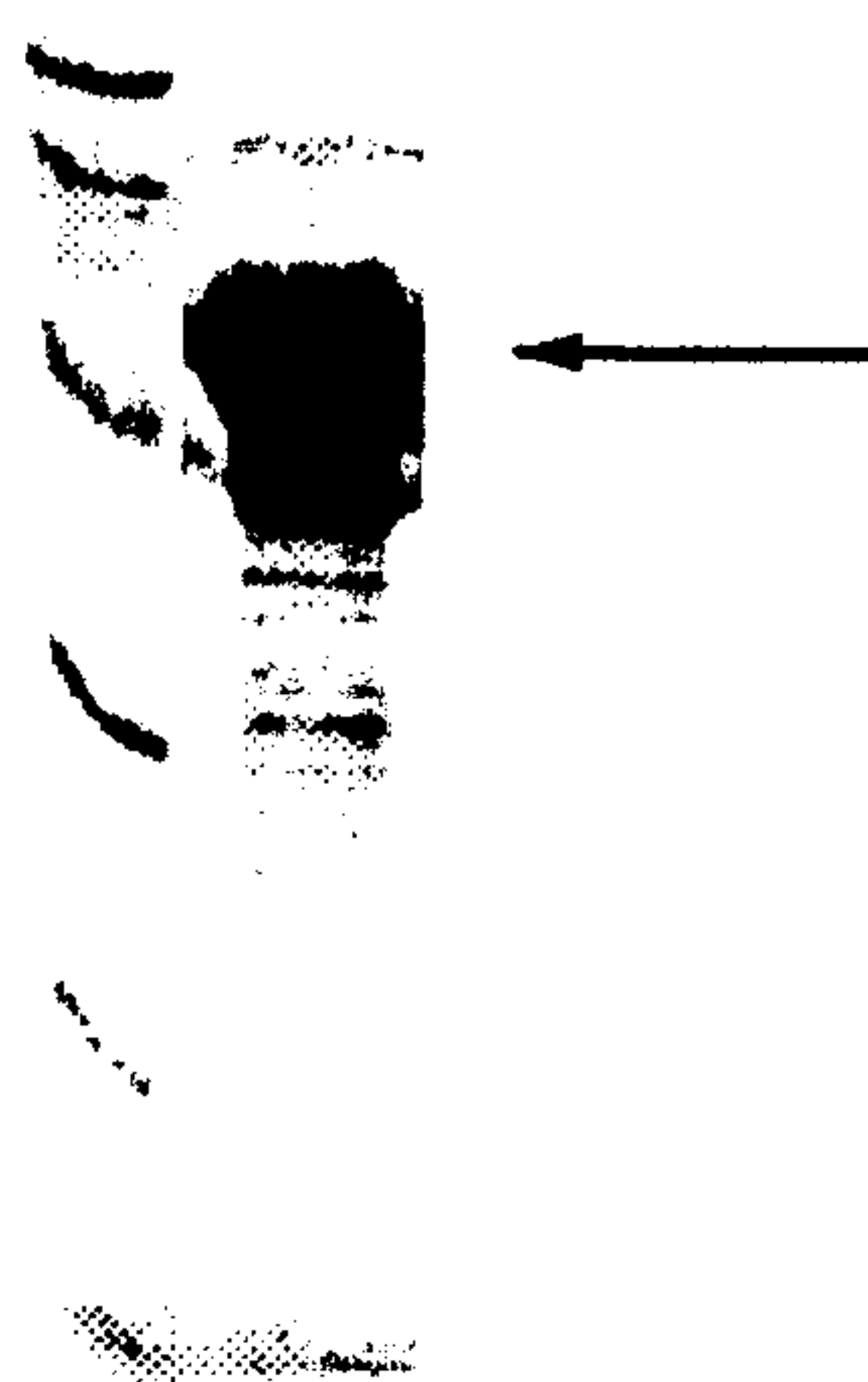
ELISA assay: positive

FIG. 3A

576 (27.8 kDa)

PURIFICATION

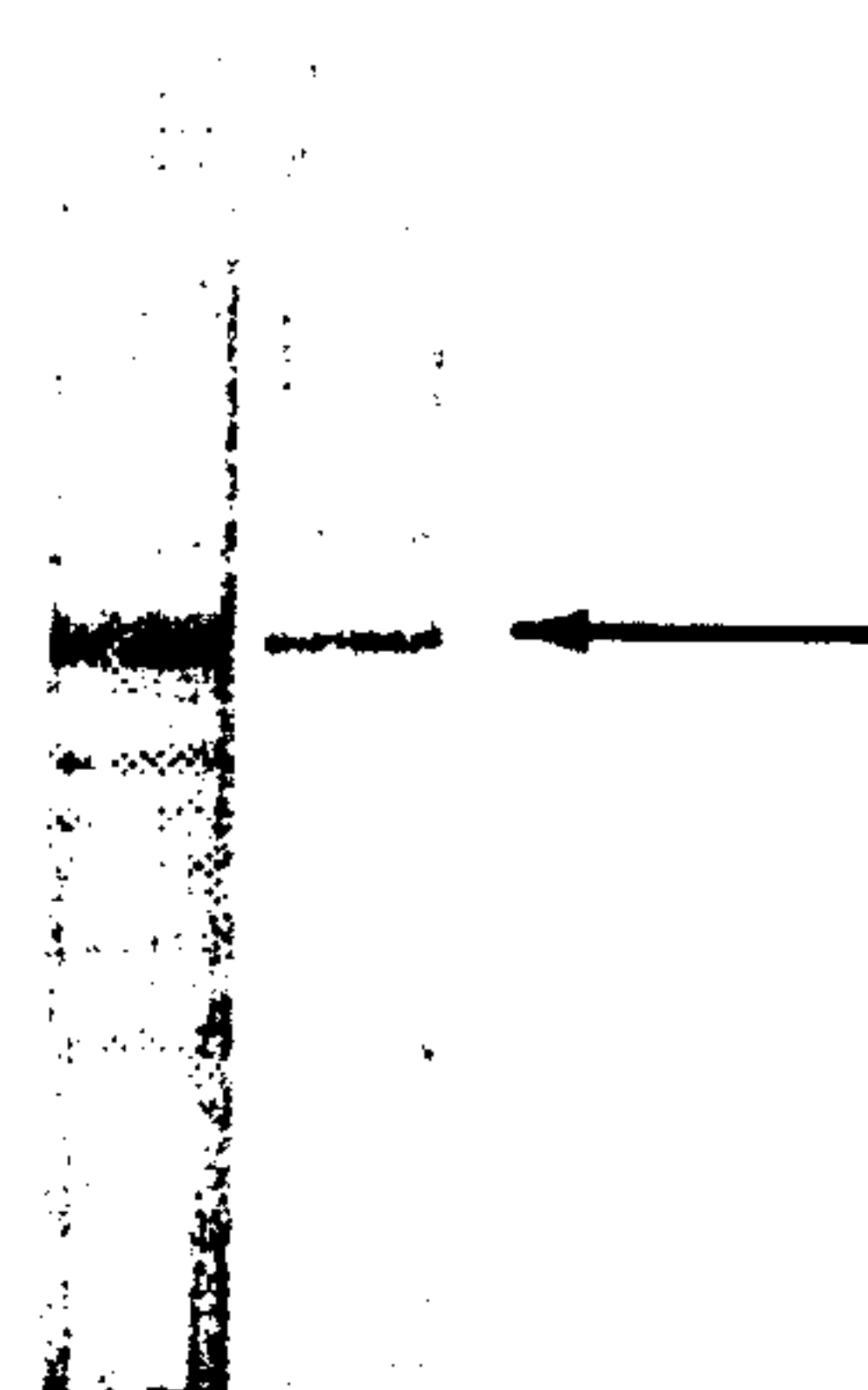
M1 576

*FIG. 3B*

576 (27.8 kDa)

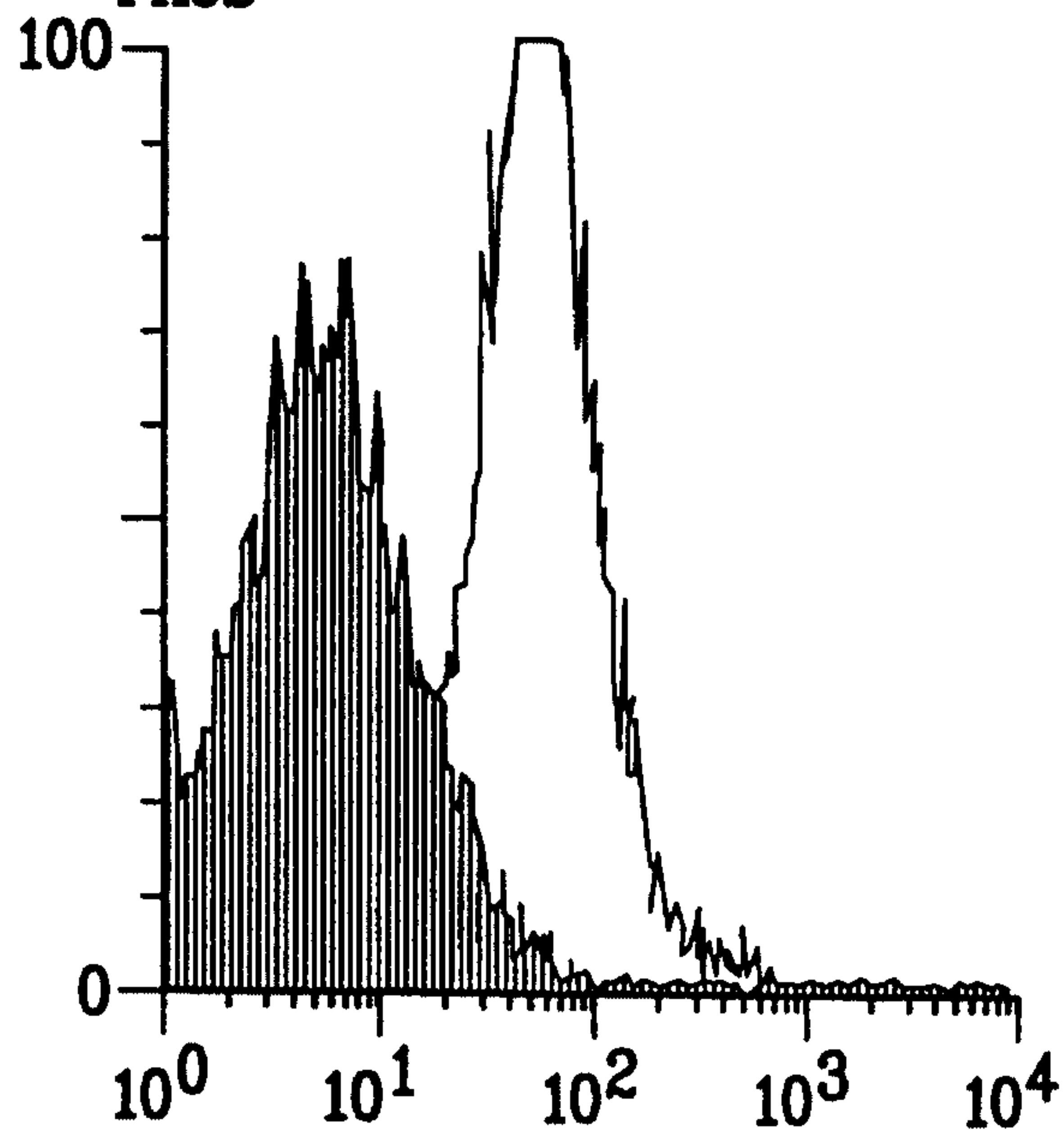
WESTERN BLOT

TP OMV

*FIG. 3C*

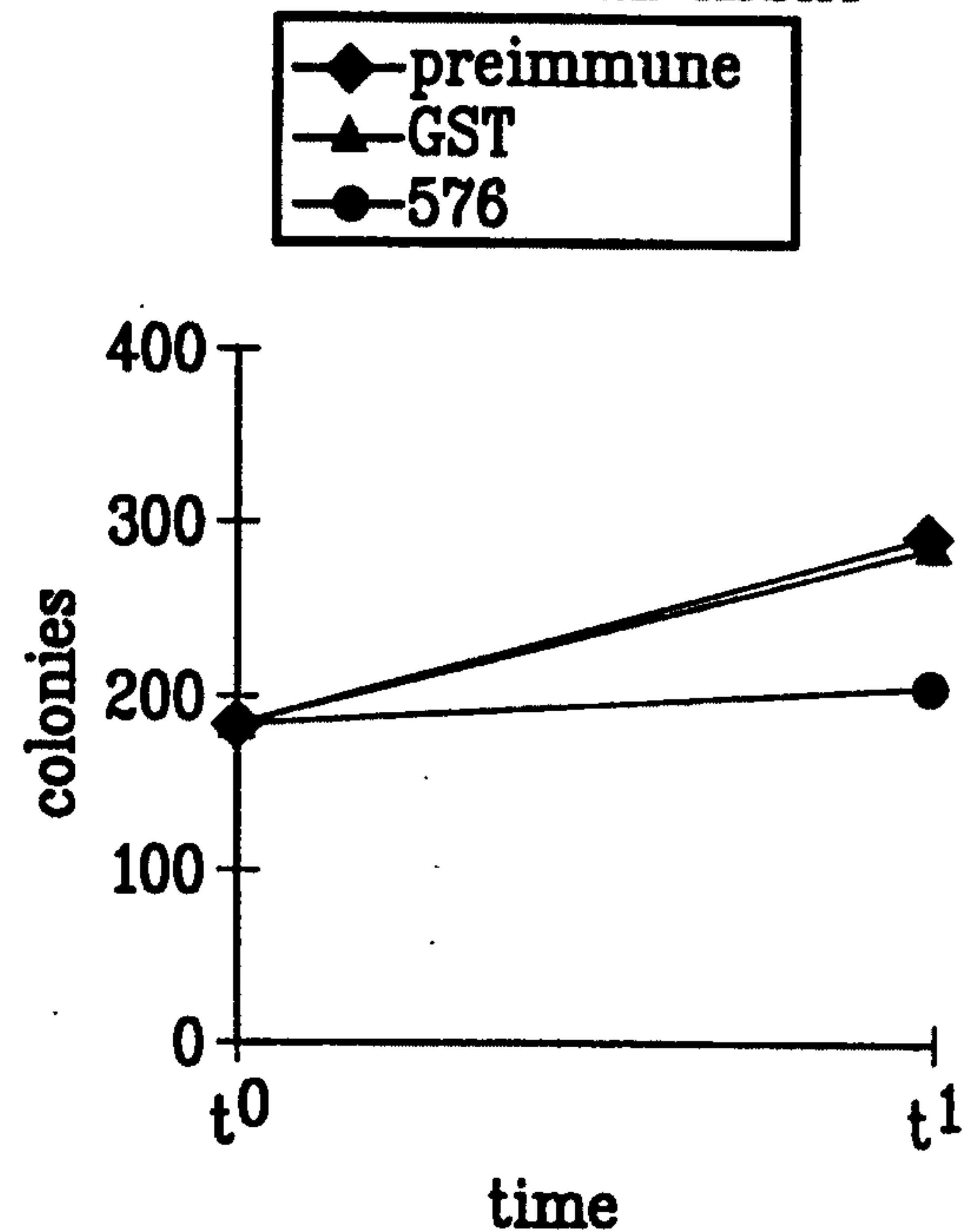
576 (27.8 kDa)

FACS

*FIG. 3D*

576 (27.8 kDa)

BACTERICIDAL ASSAY

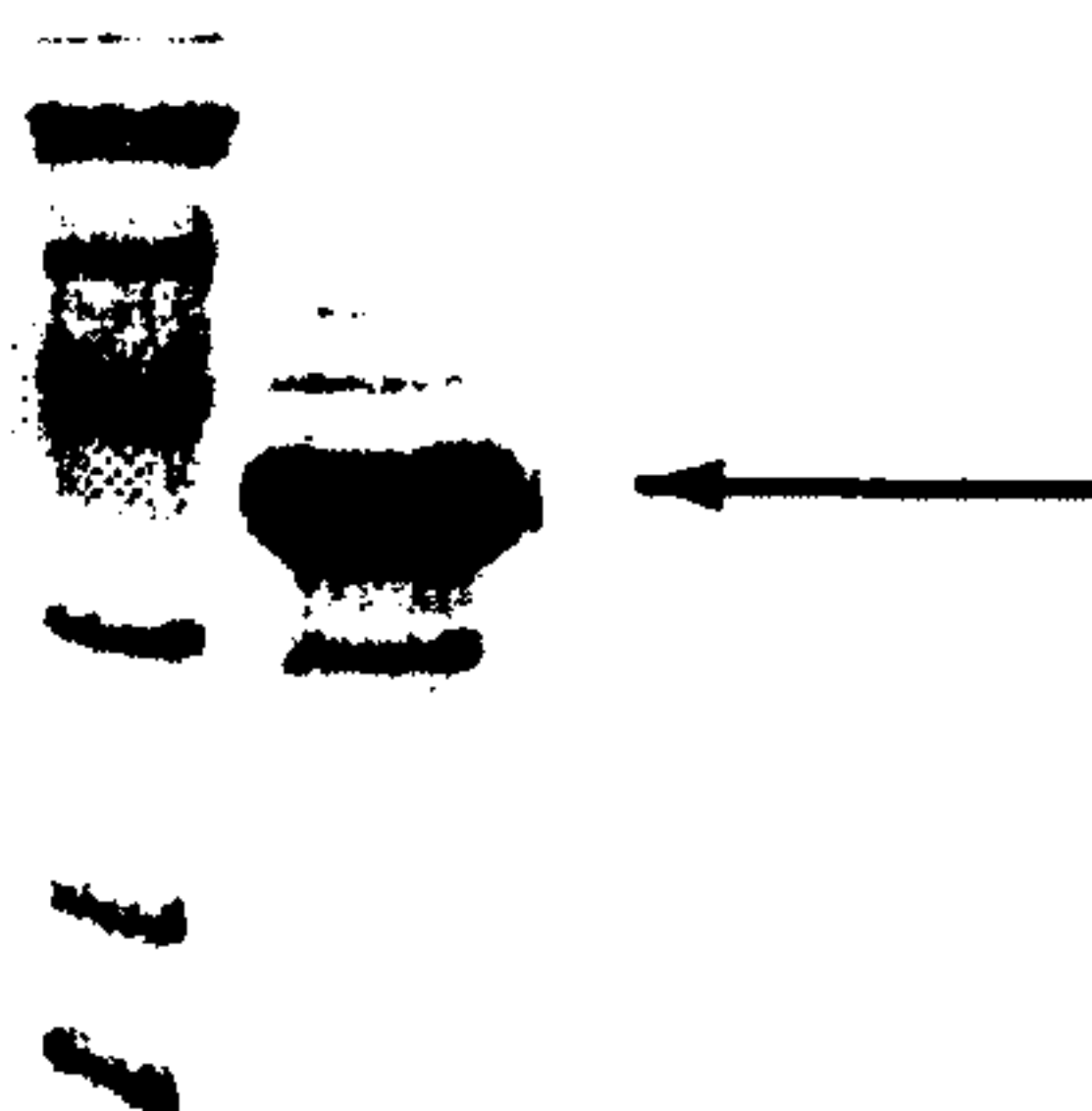
*FIG. 3E*

576 (27.8 kDa)

ELISA assay: positive

FIG. 4A

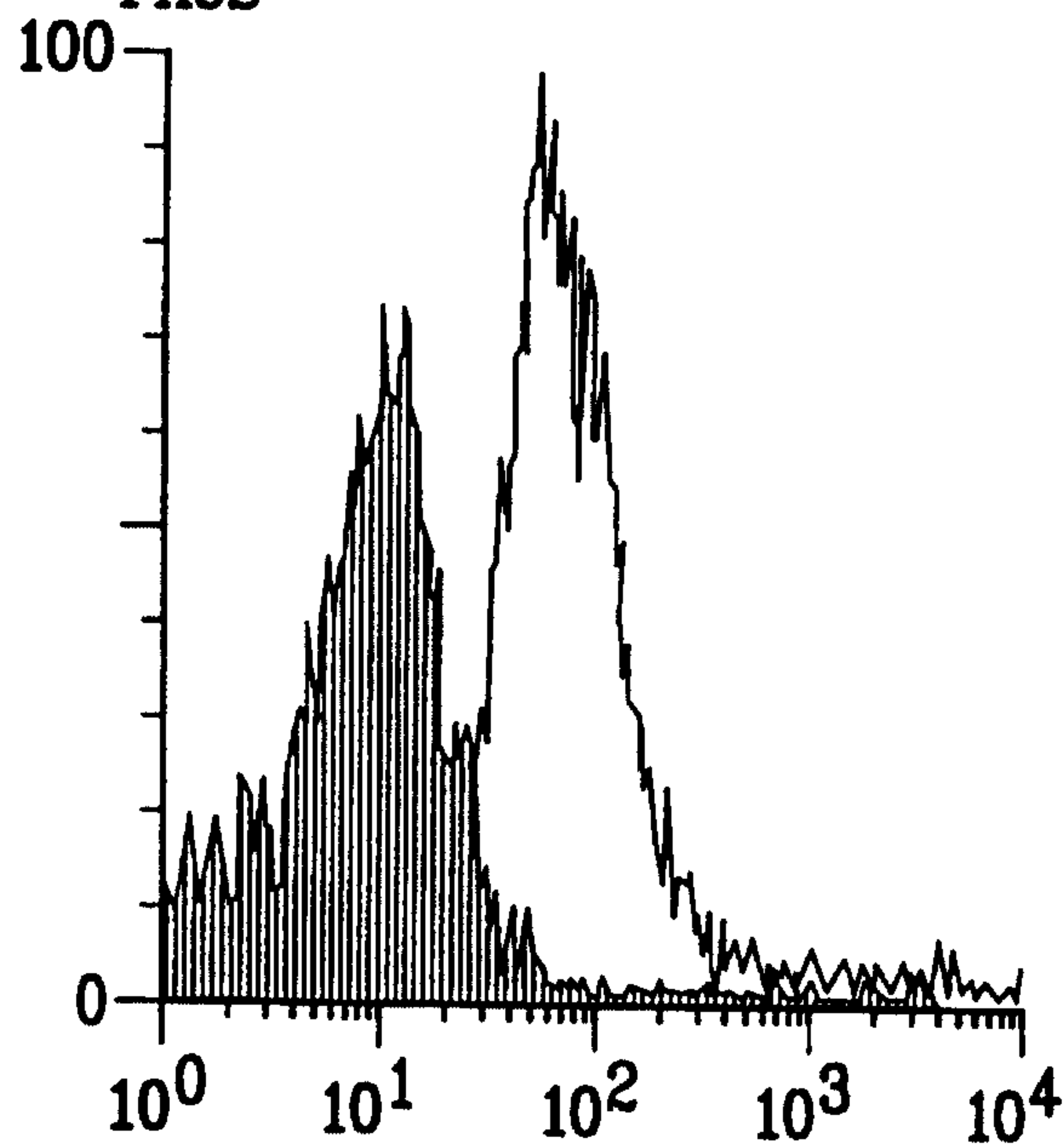
519 (33 kDa)
PURIFICATION
M1 519

*FIG. 4B*

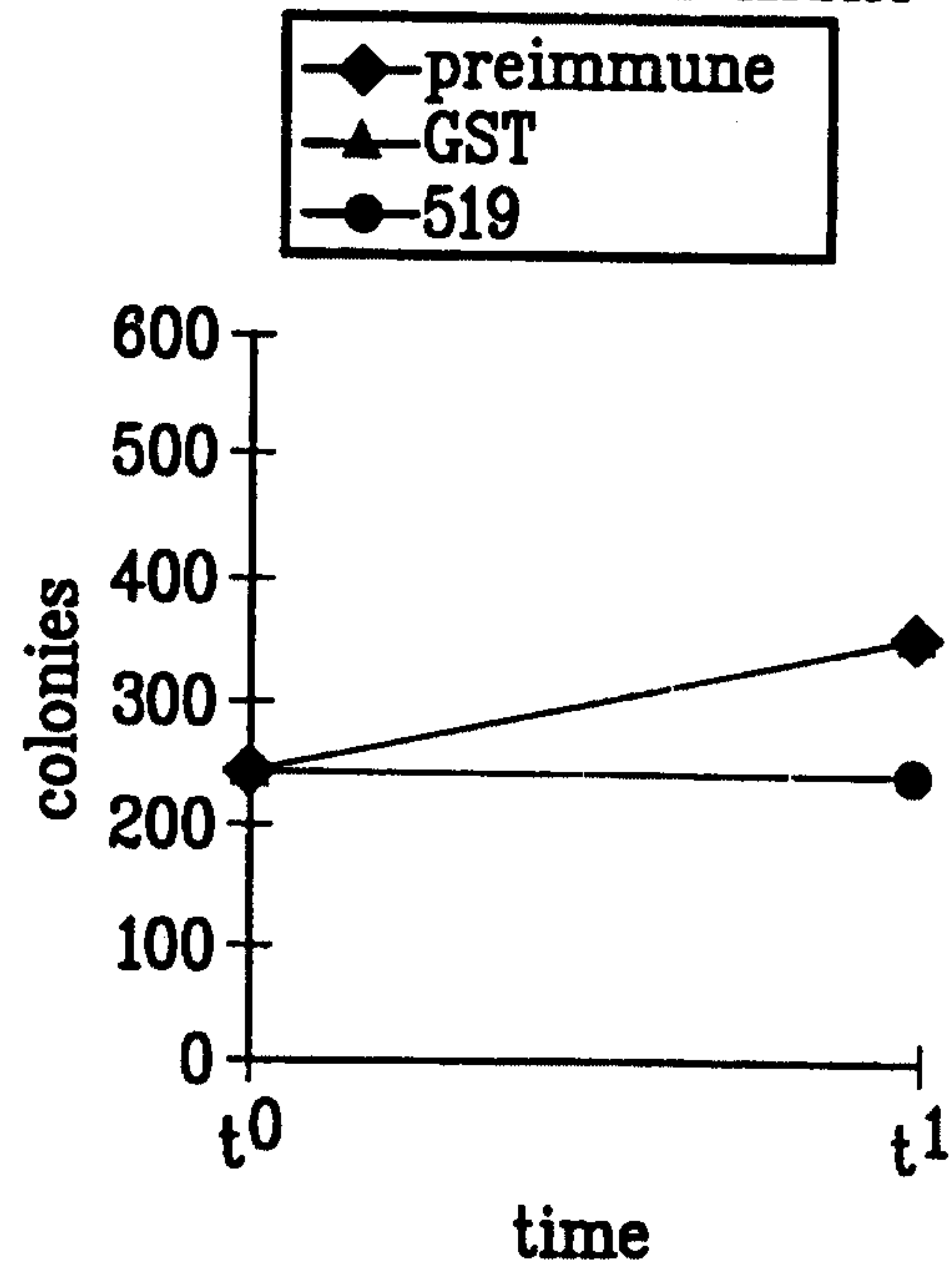
519 (33 kDa)
WESTERN BLOT
TP OMV

*FIG. 4C*

519 (33 kDa)
FACS

*FIG. 4D*

519 (33 kDa)
BACTERICIDAL ASSAY

*FIG. 4E*

519 (33 kDa)
ELISA assay: positive

FIG. 5A

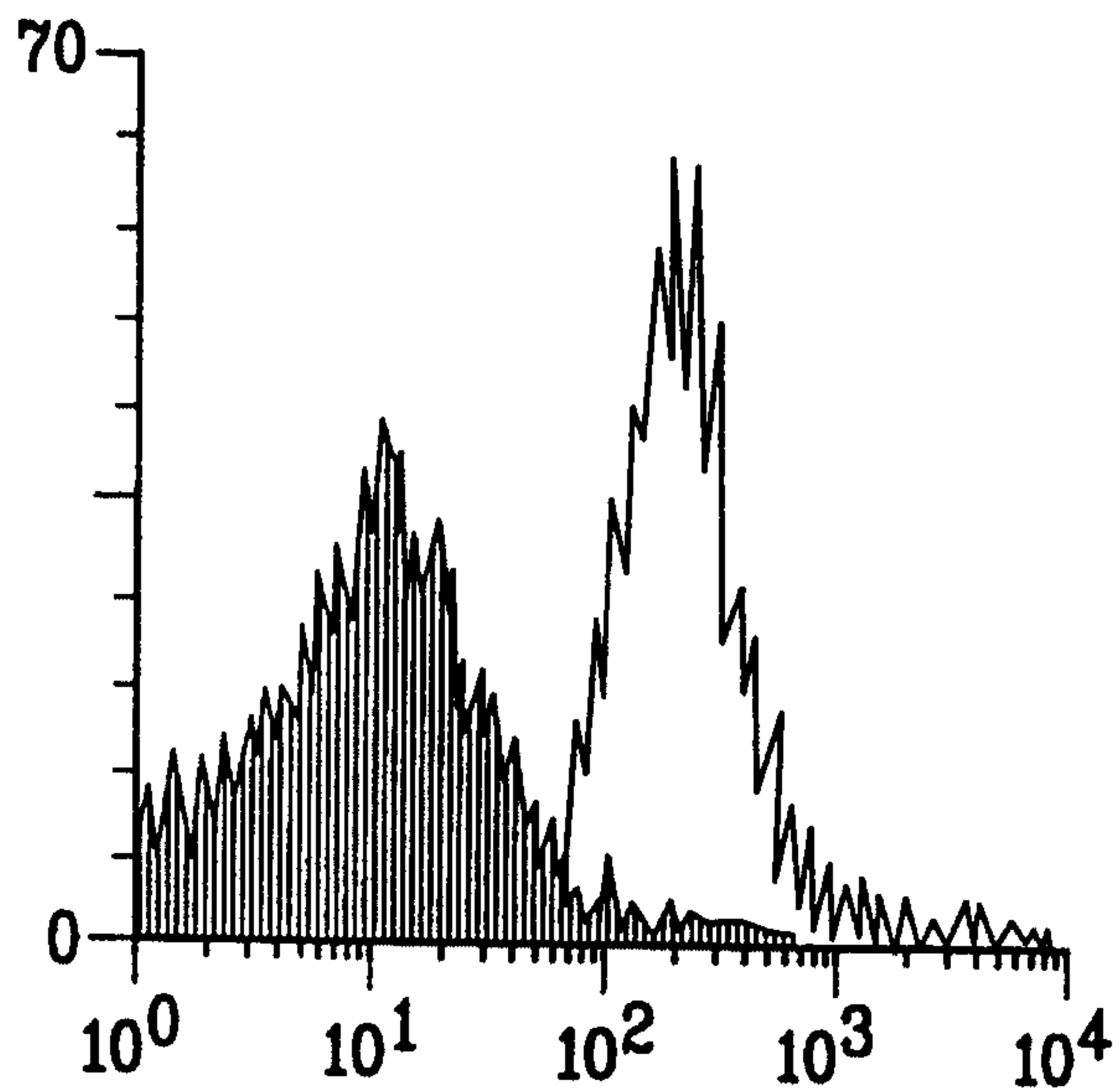
121 (40 kDa)
PURIFICATION
M1 121

*FIG. 5B*

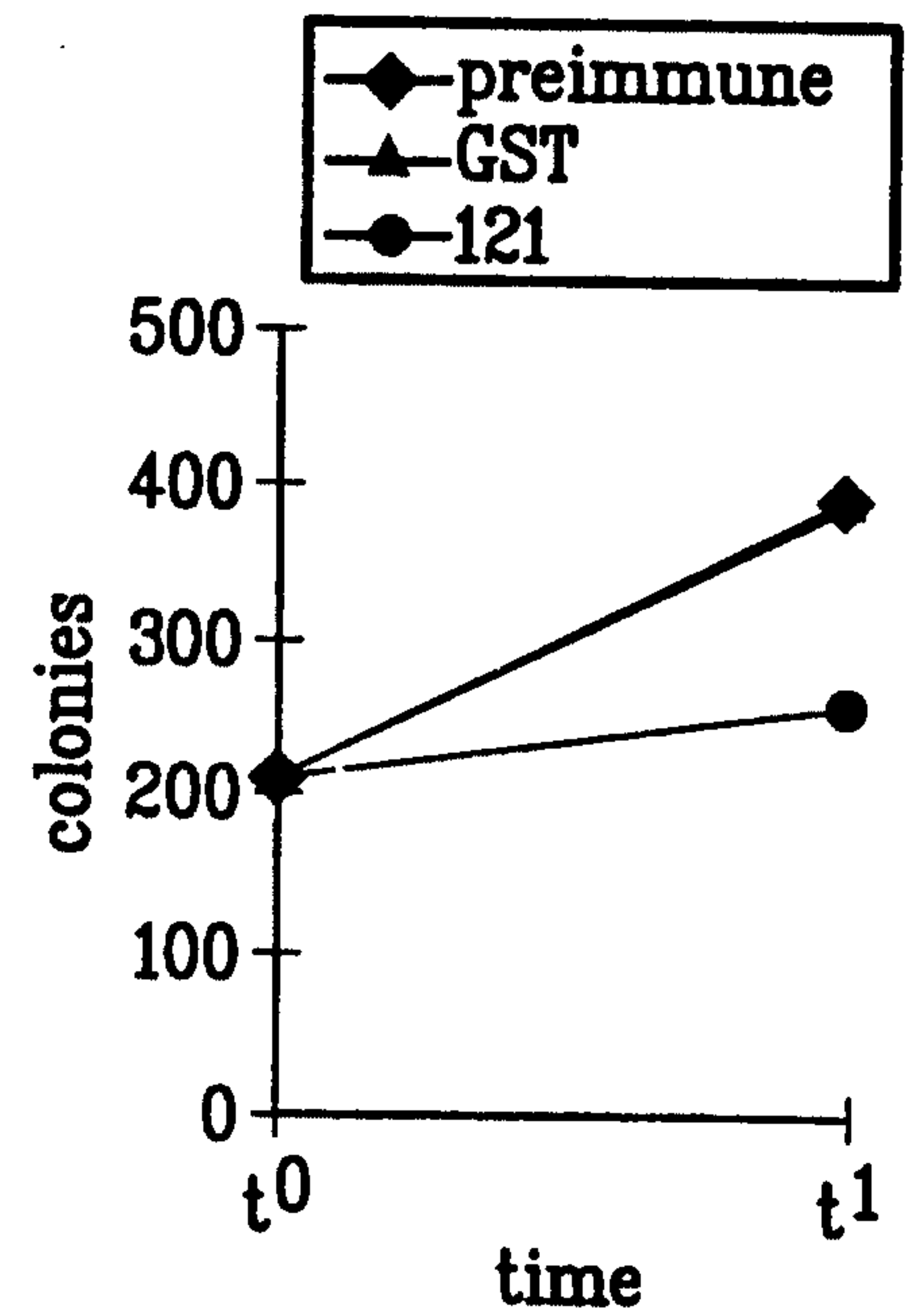
121 (40 kDa)
WESTERN BLOT
TP OMV

*FIG. 5C*

121 (40 kDa)
FACS

*FIG. 5D*

121 (40 kDa)
BACTERICIDAL ASSAY

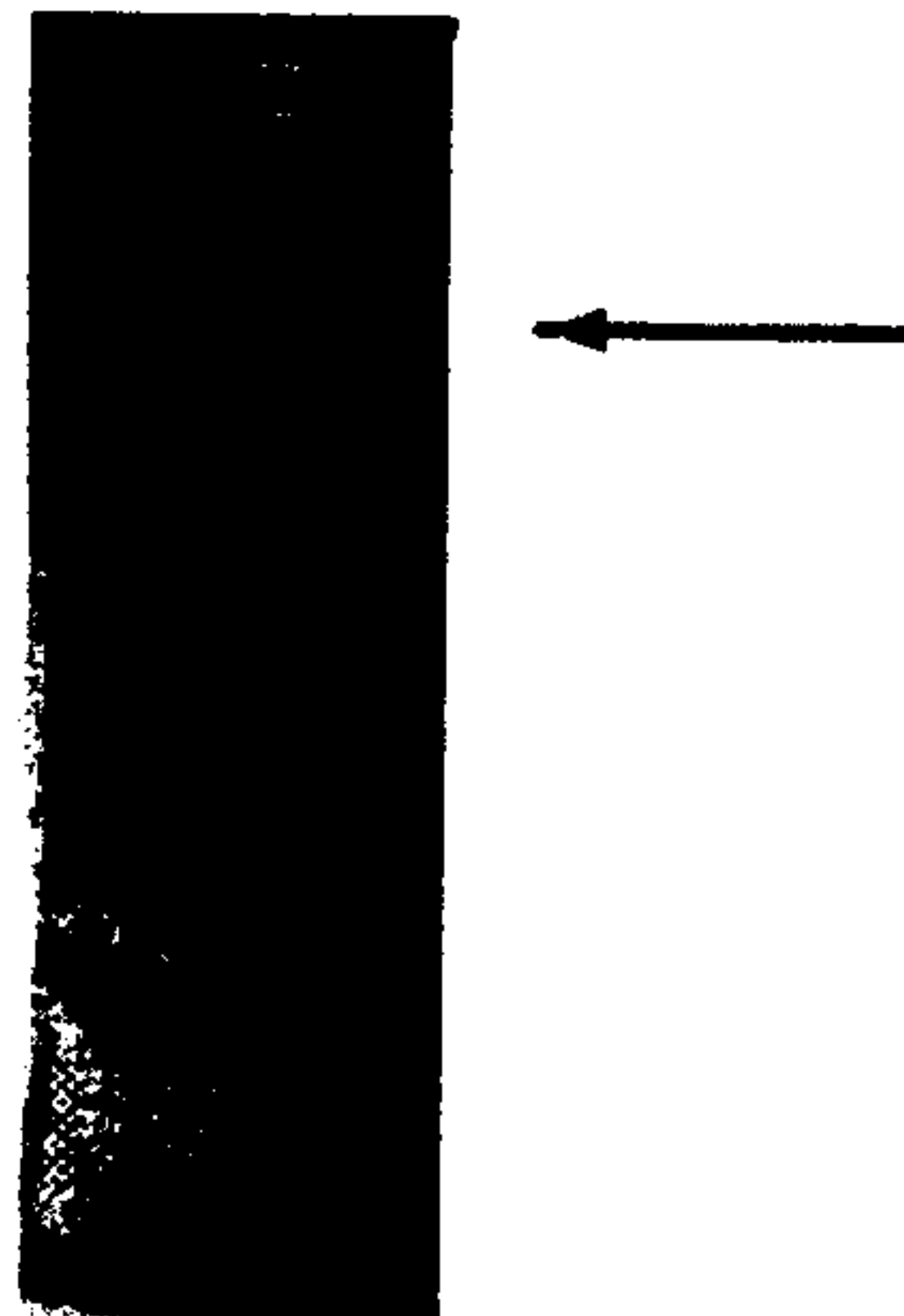
*FIG. 5E*

121 (40 kDa)

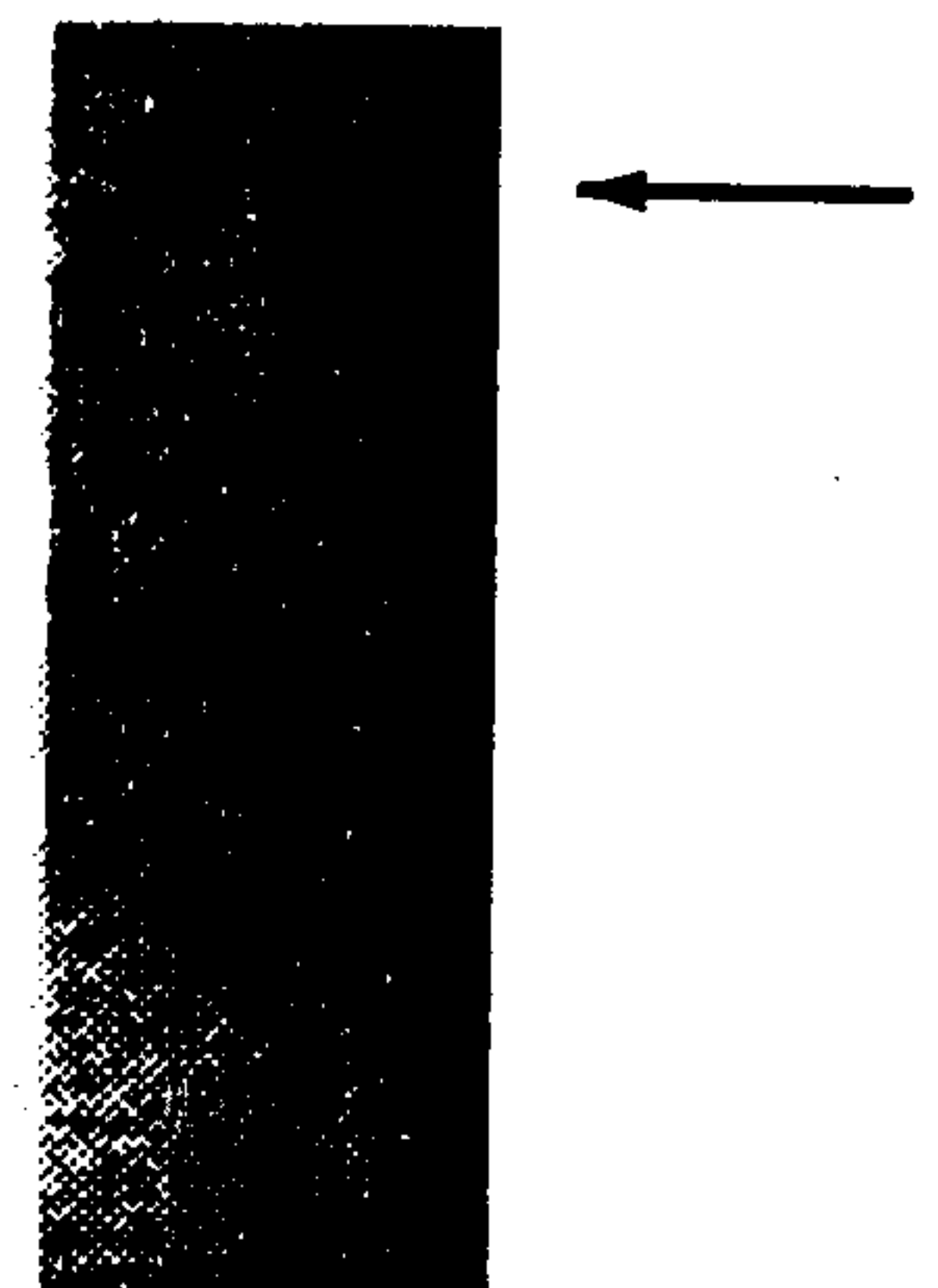
ELISA assay: positive

FIG. 6A

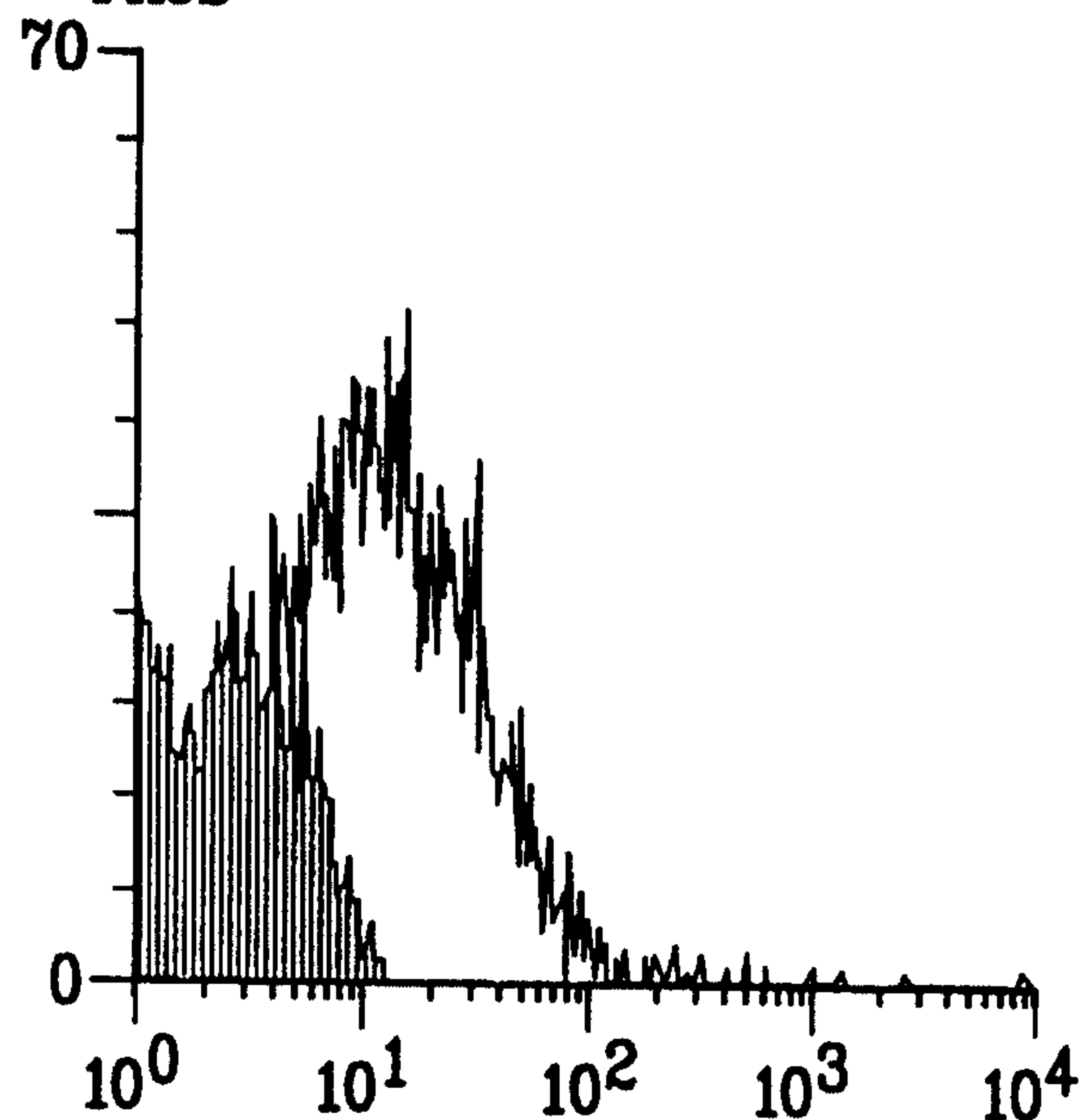
128 (101 kDa)
PURIFICATION
M1 128

*FIG. 6B*

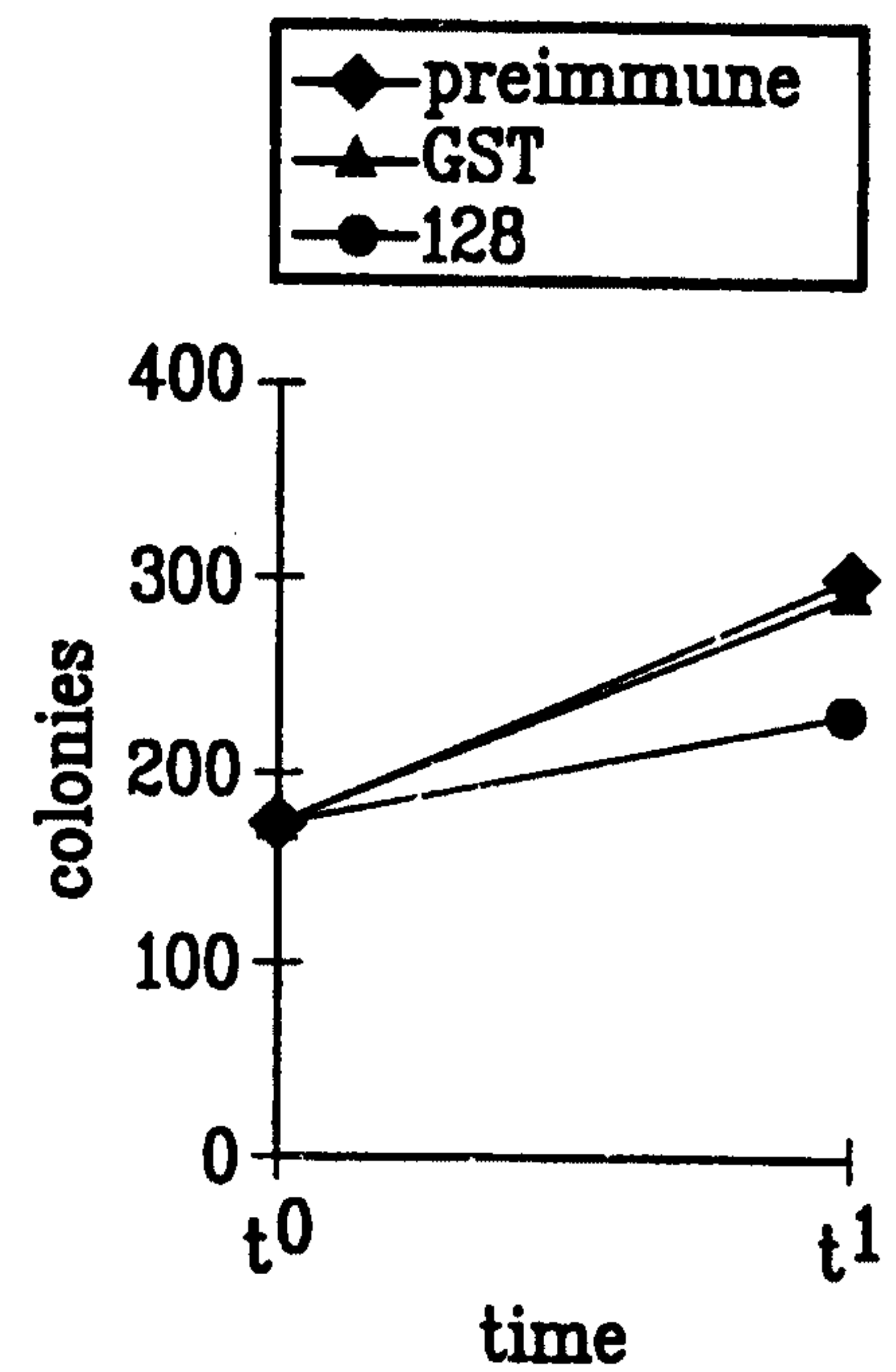
128 (101 kDa)
WESTERN BLOT
TP OMV

*FIG. 6C*

128 (101 kDa)
FACS

*FIG. 6D*

128 (101 kDa)
BACTERICIDAL ASSAY

*FIG. 6E*

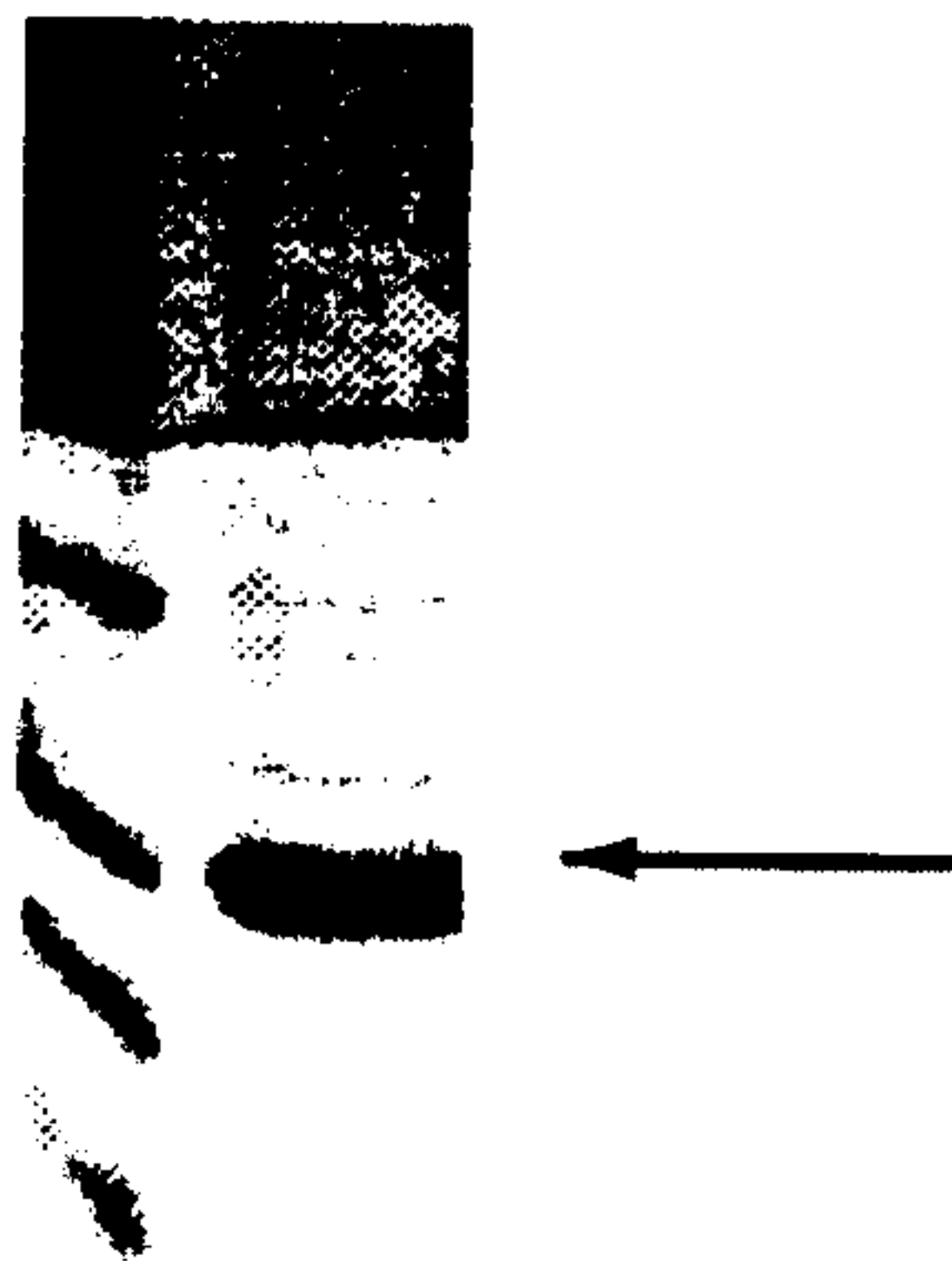
128 (101 kDa)
ELISA assay: positive

FIG. 7A

206 (17 kDa)

PURIFICATION

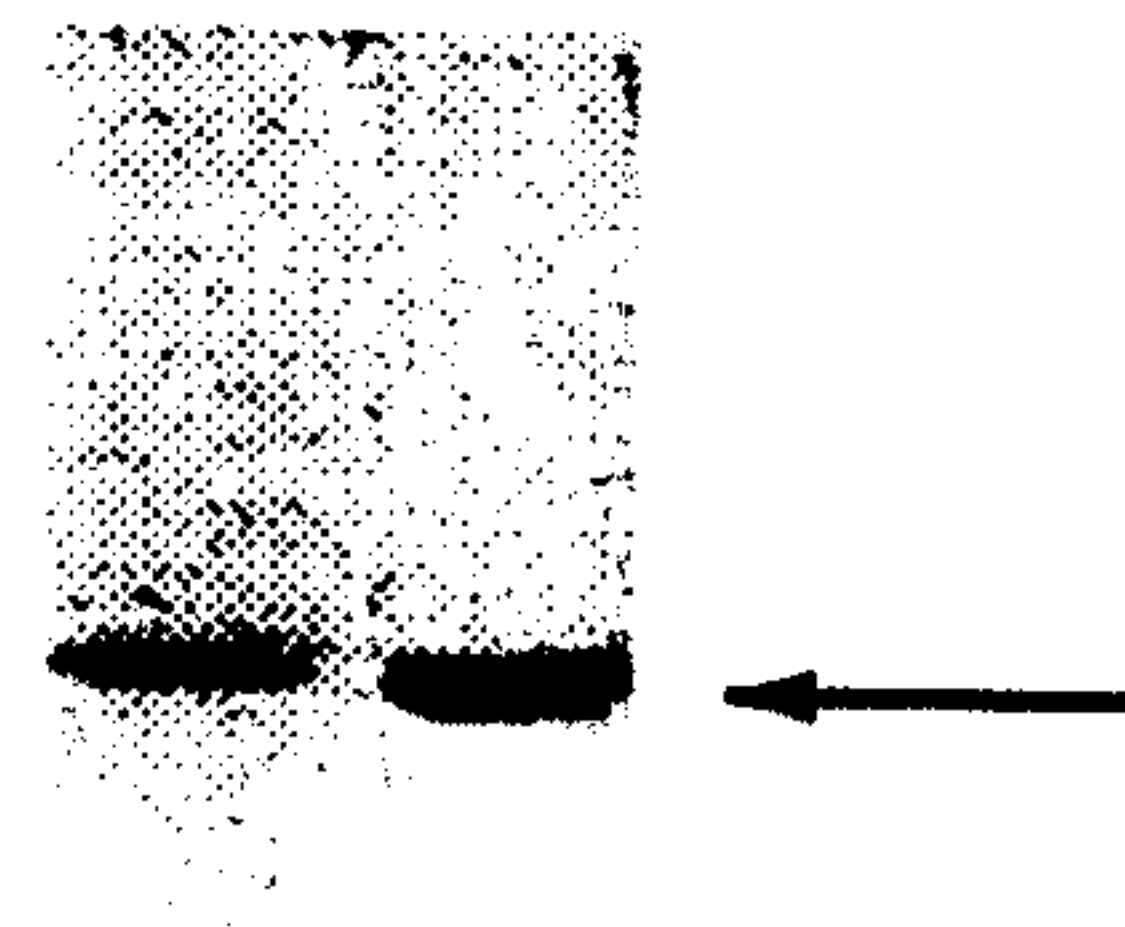
M1 206

*FIG. 7B*

206 (17 kDa)

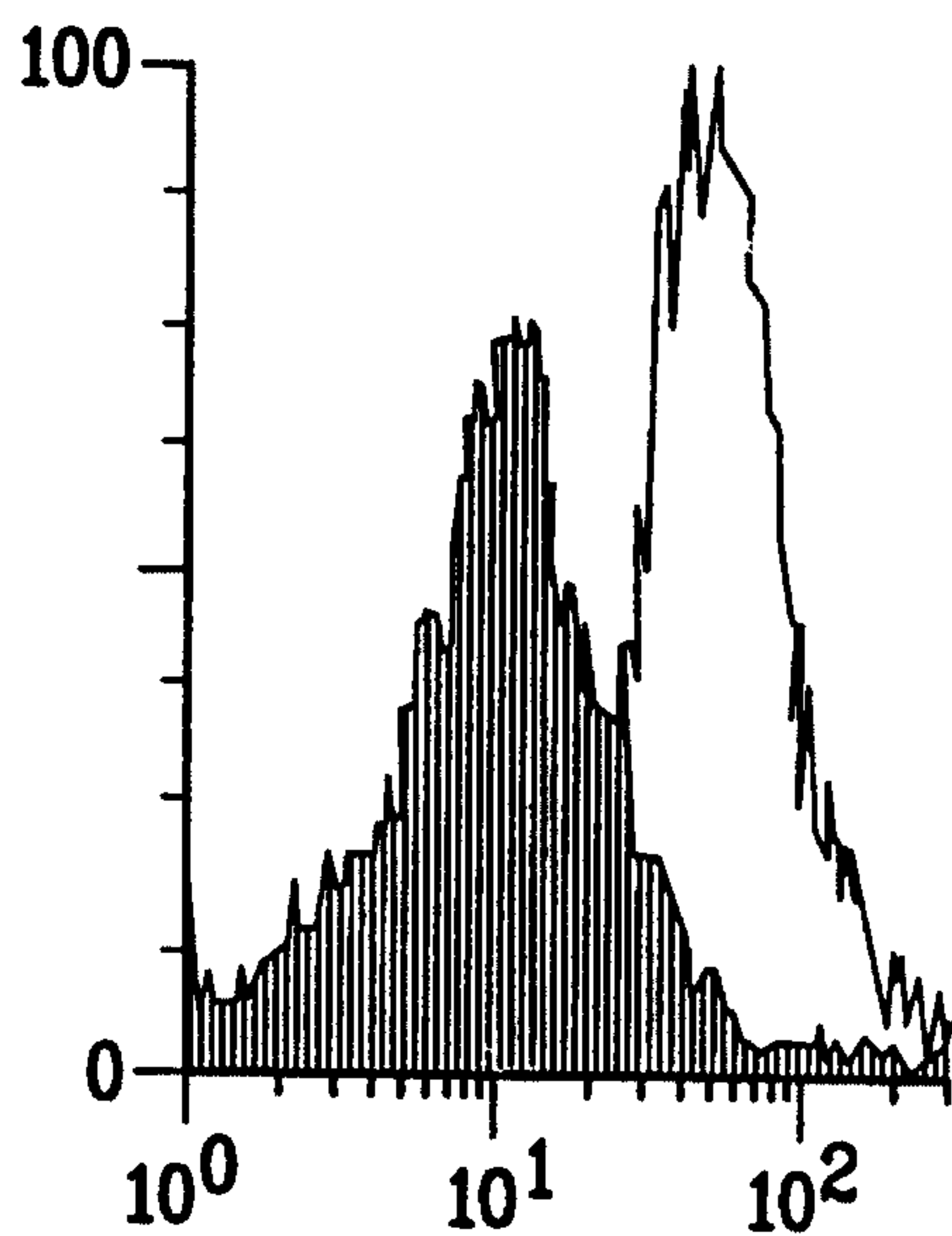
WESTERN BLOT

TP OMV

*FIG. 7C*

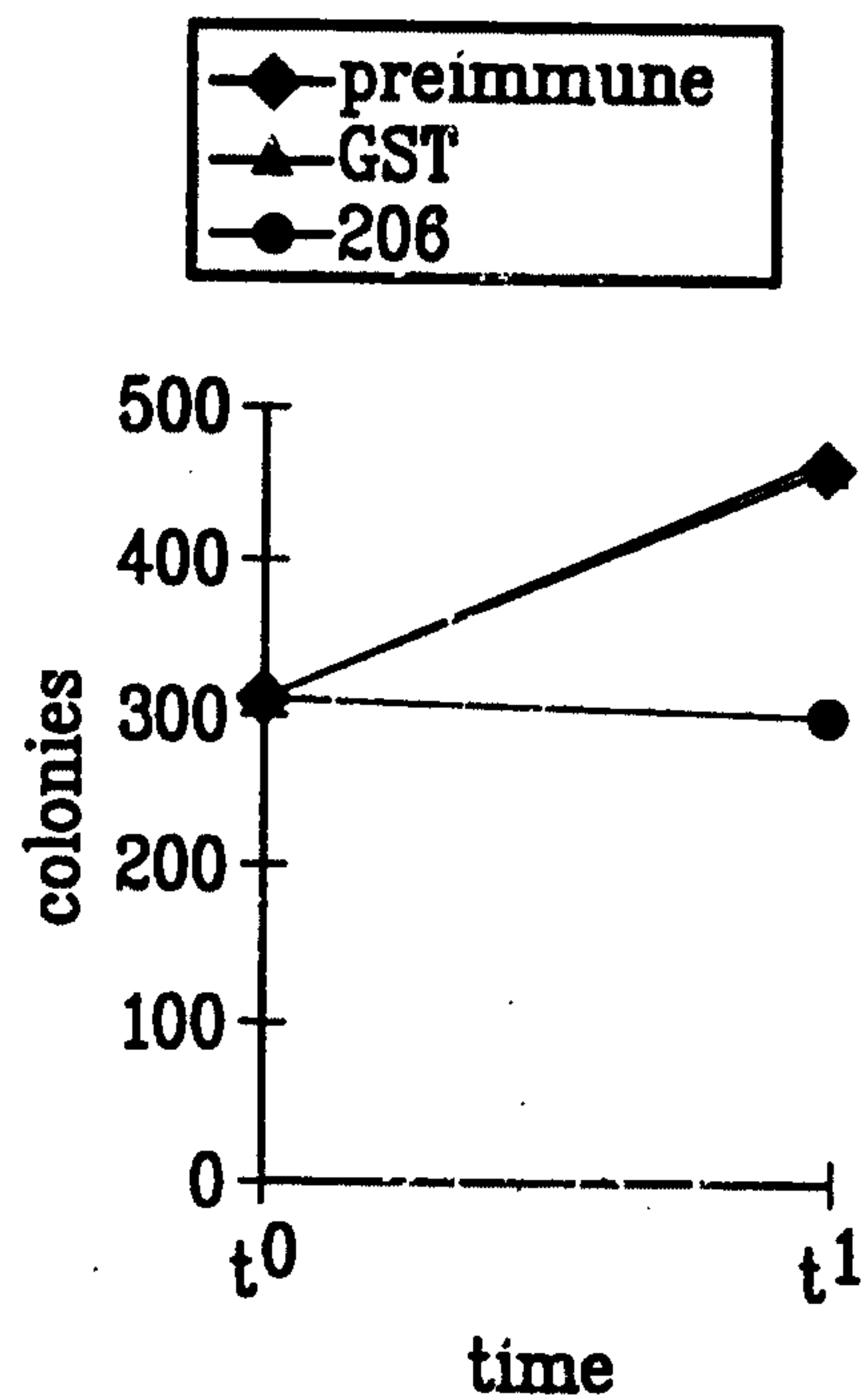
206 (17 kDa)

FACS

*FIG. 7D*

206 (17 kDa)

BACTERICIDAL ASSAY

*FIG. 7E*

206 (17 kDa)

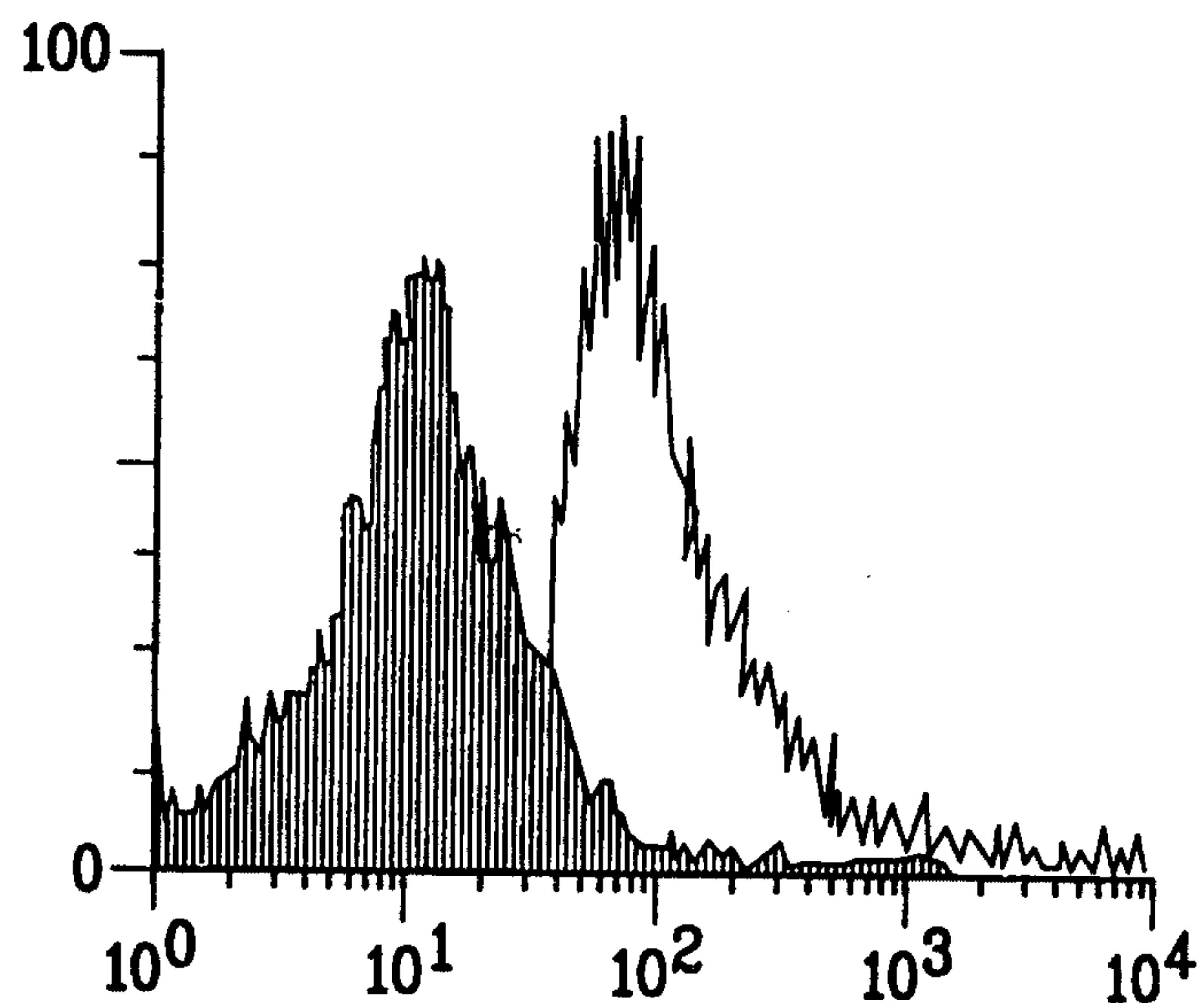
ELISA assay: positive

FIG. 8A

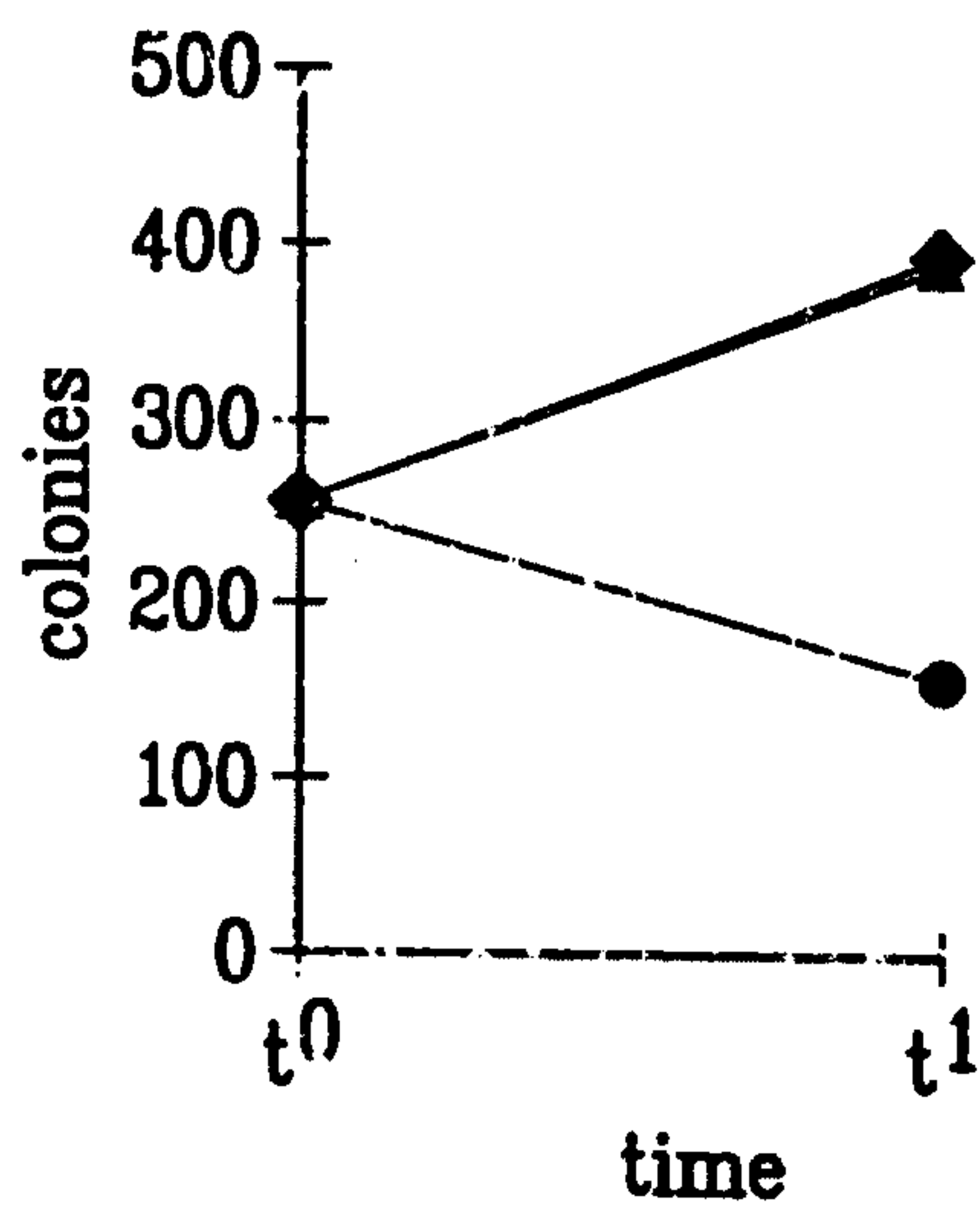
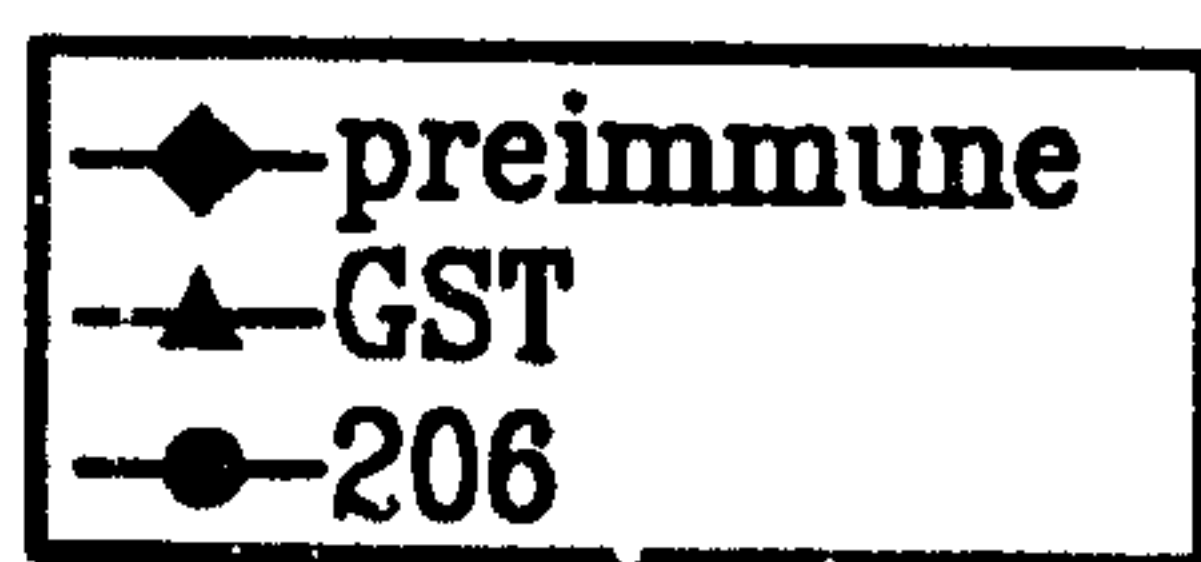
287 (78 kDa)
PURIFICATION
M1 287

*FIG. 8B*

287 (78 kDa)
FACS

*FIG. 8C*

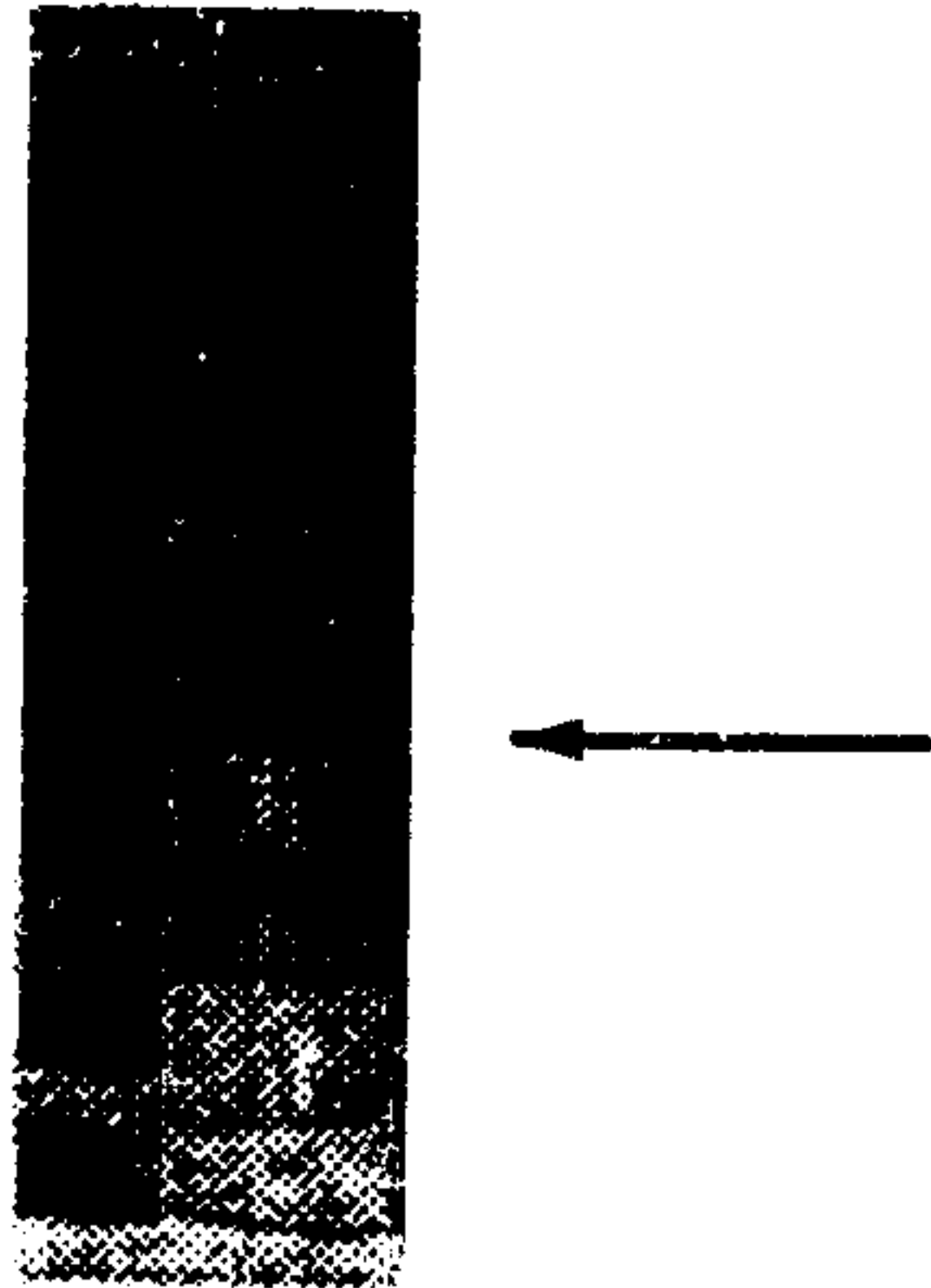
287 (78 kDa)
BACTERICIDAL ASSAY

*FIG. 8D*

287 (78 kDa)
ELISA assay: positive

FIG. 9A

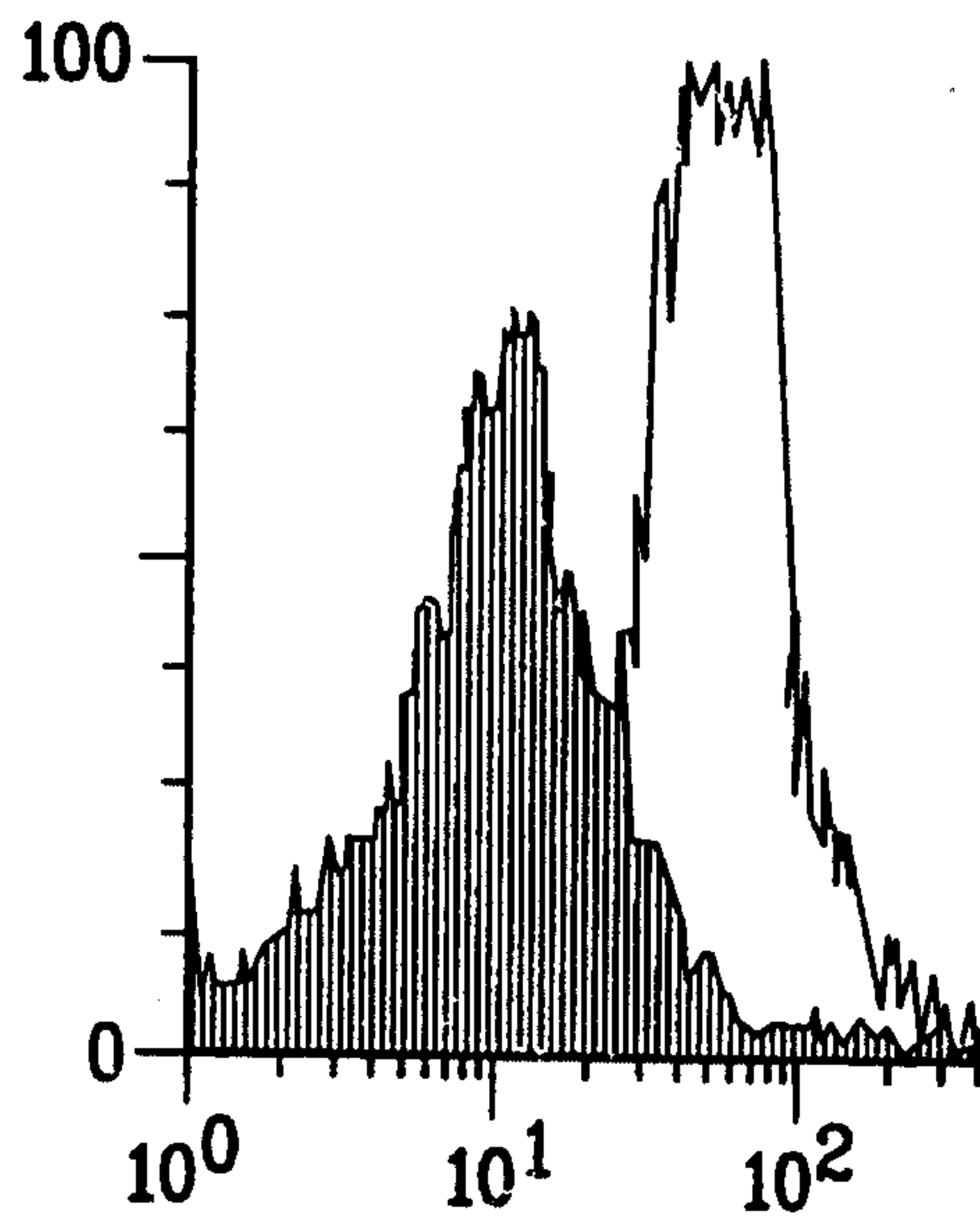
406 (33 kDa)
PURIFICATION
M1 406

*FIG. 9B*

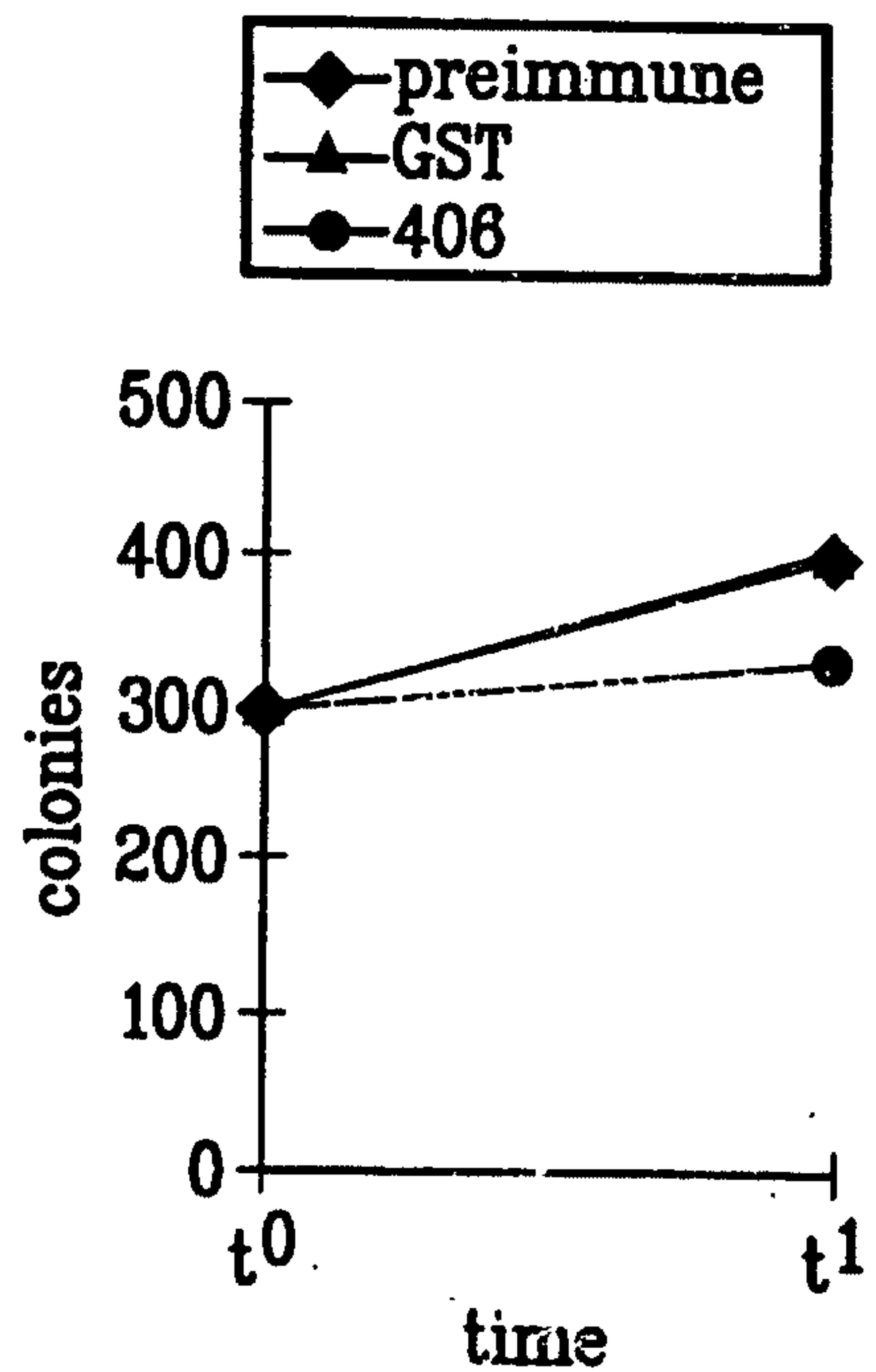
406 (33 kDa)
WESTERN BLOT
TP OMV

*FIG. 9C*

406 (33 kDa)
FACS

*FIG. 9D*

406 (33 kDa)
BACTERICIDAL ASSAY

*FIG. 9E*

406 (33 kDa)
ELISA assay: positive

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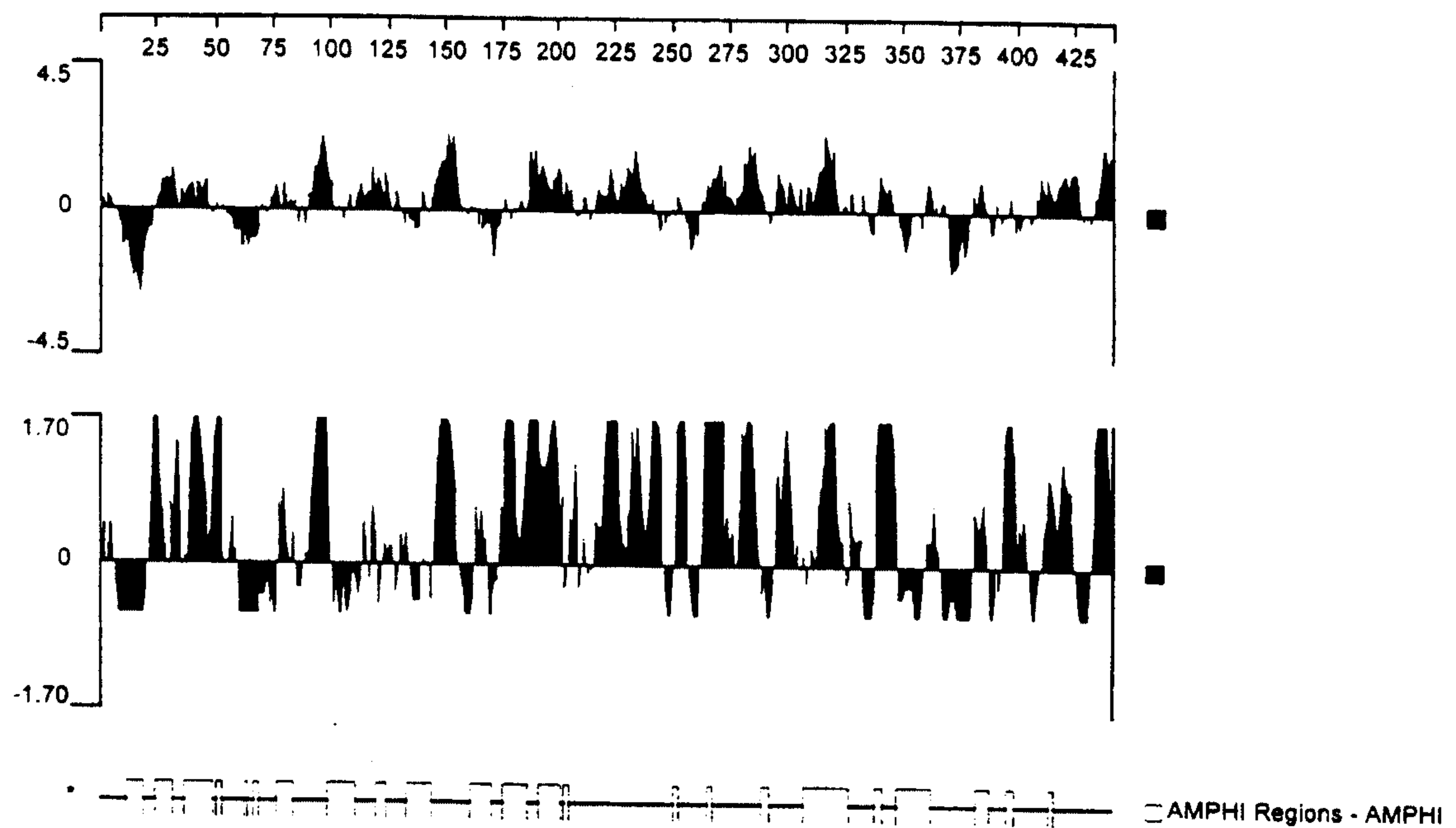
919Hydrophilicity Plot, Antigenic Index and AMPHI Regions

Fig. 10

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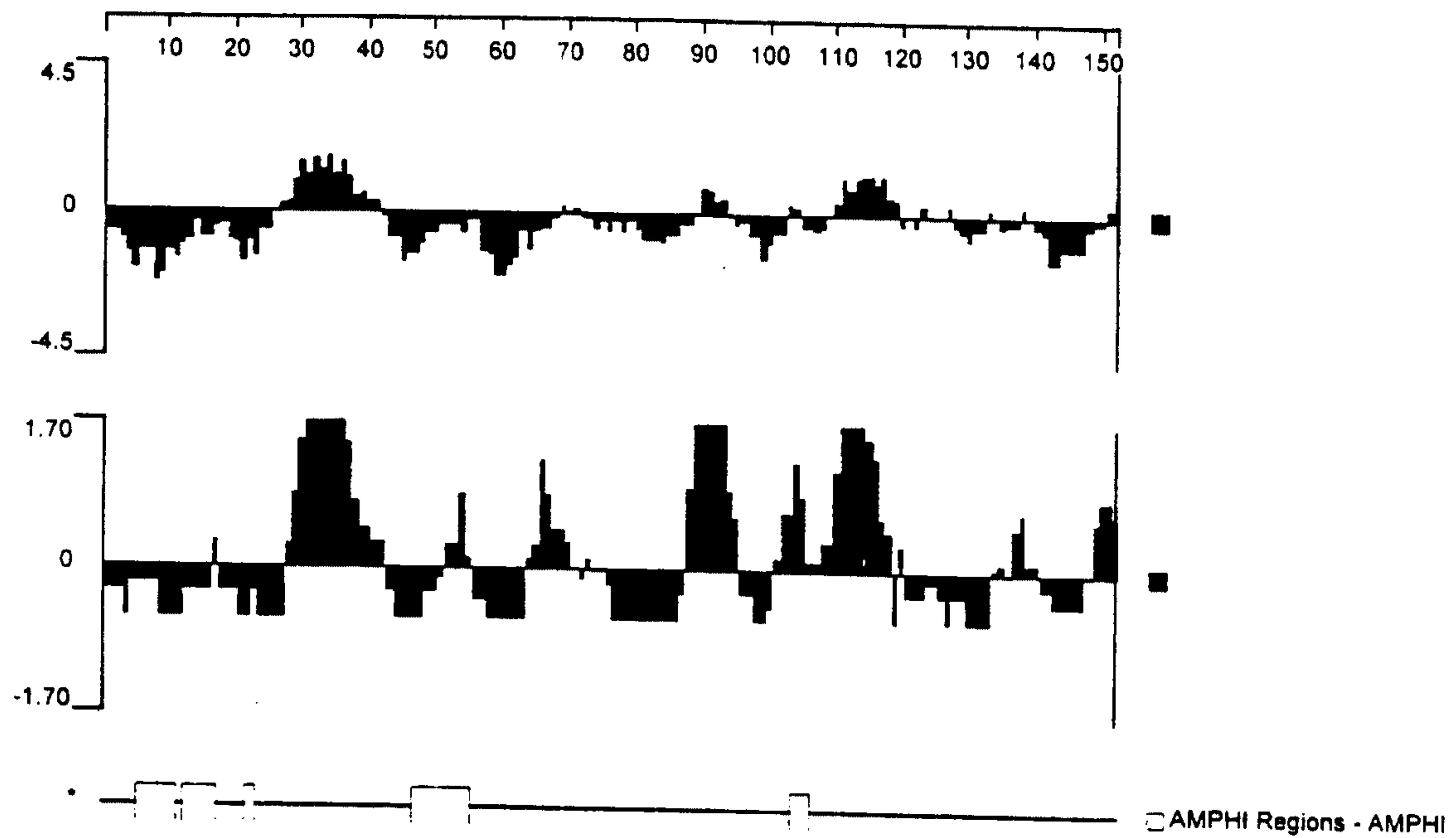
279Hydrophilicity Plot, Antigenic Index and AMPHI Regions

Fig. 11

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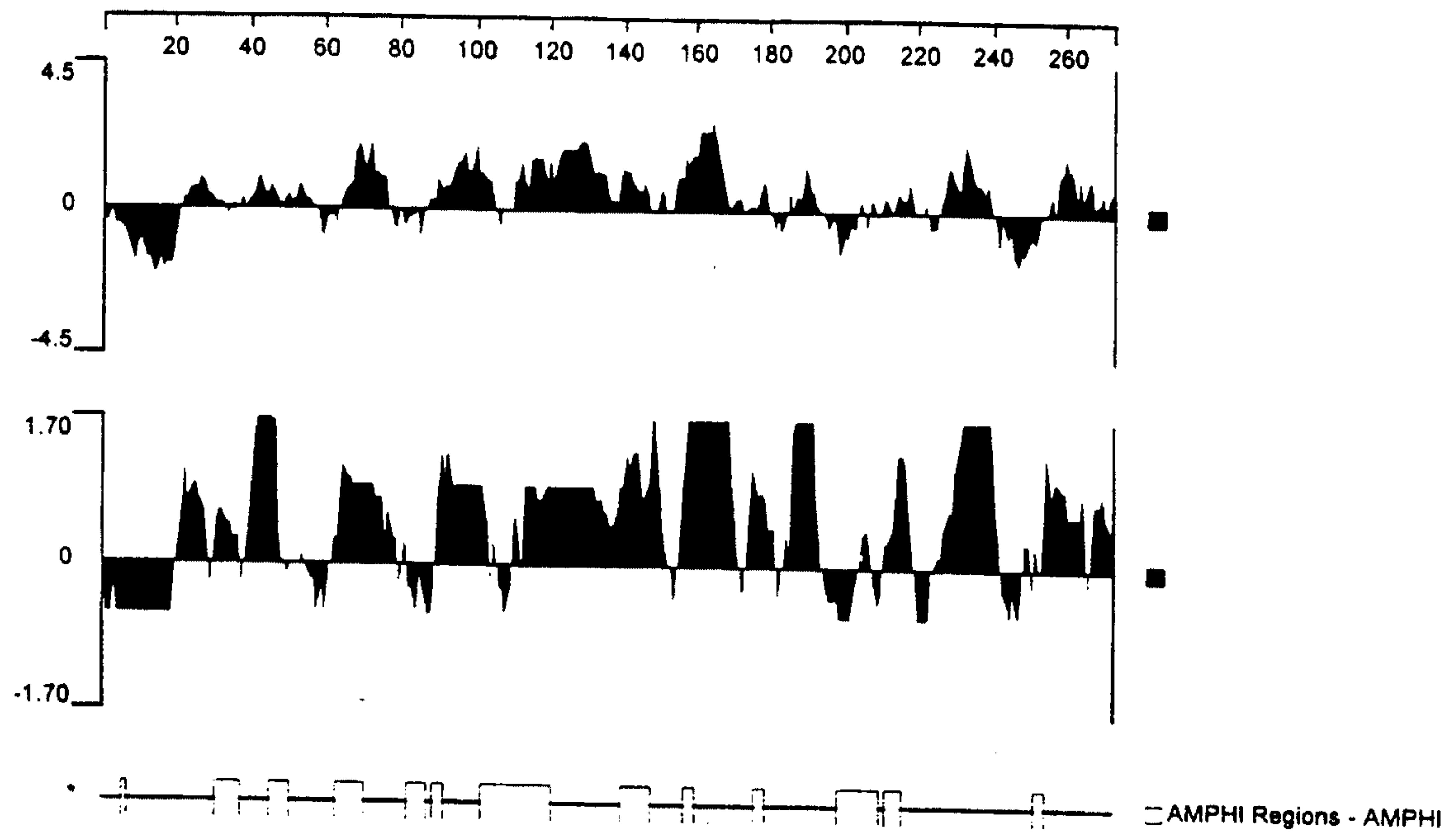
576-1Hydrophilicity Plot, Antigenic Index and AMPHI Regions

Fig. 12

519-1
Hydrophilicity Plot, Antigenic Index and AMPHI Regions

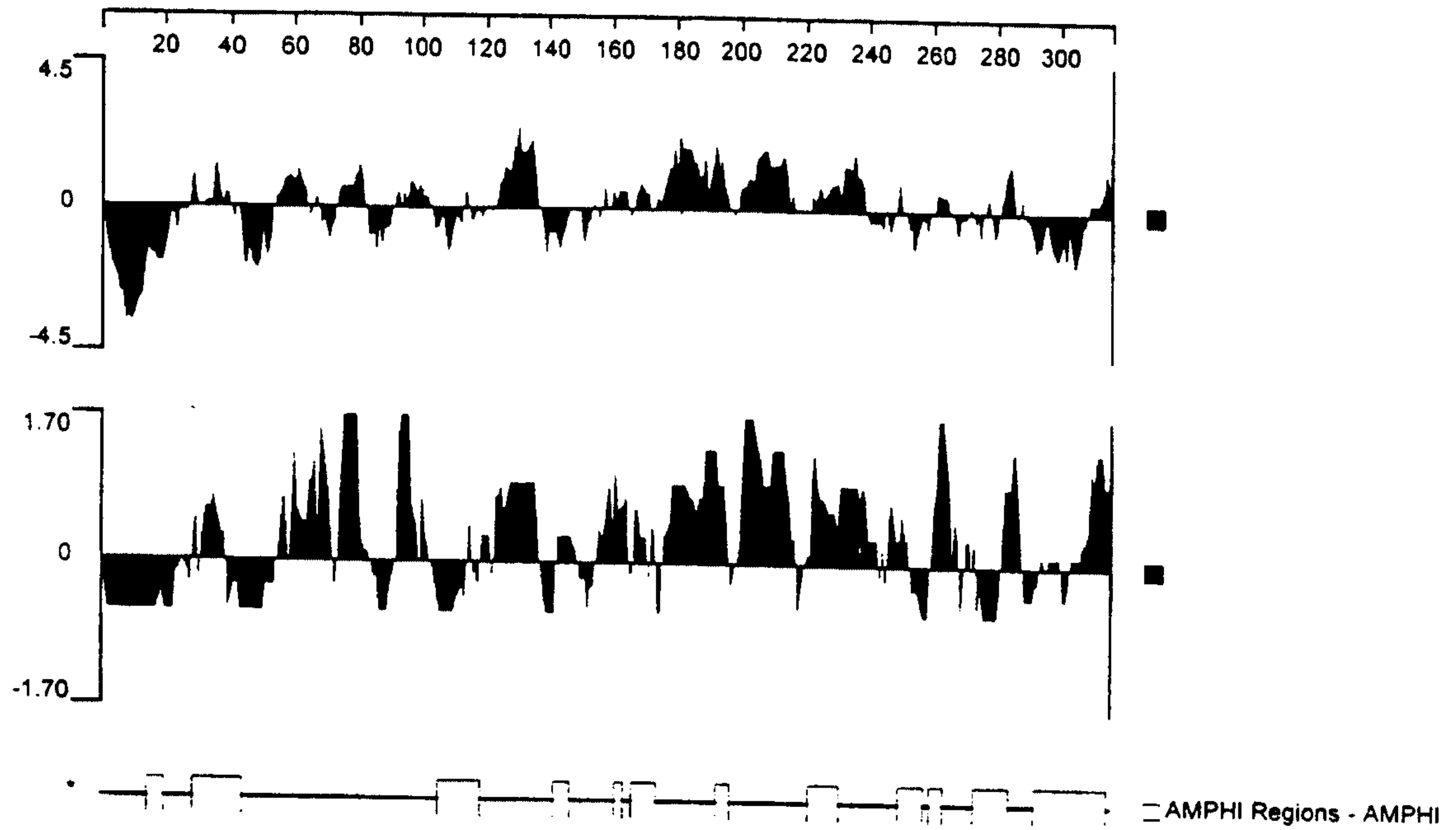


Fig. 13

121-1
Hydrophilicity Plot, Antigenic Index and AMPHI Regions

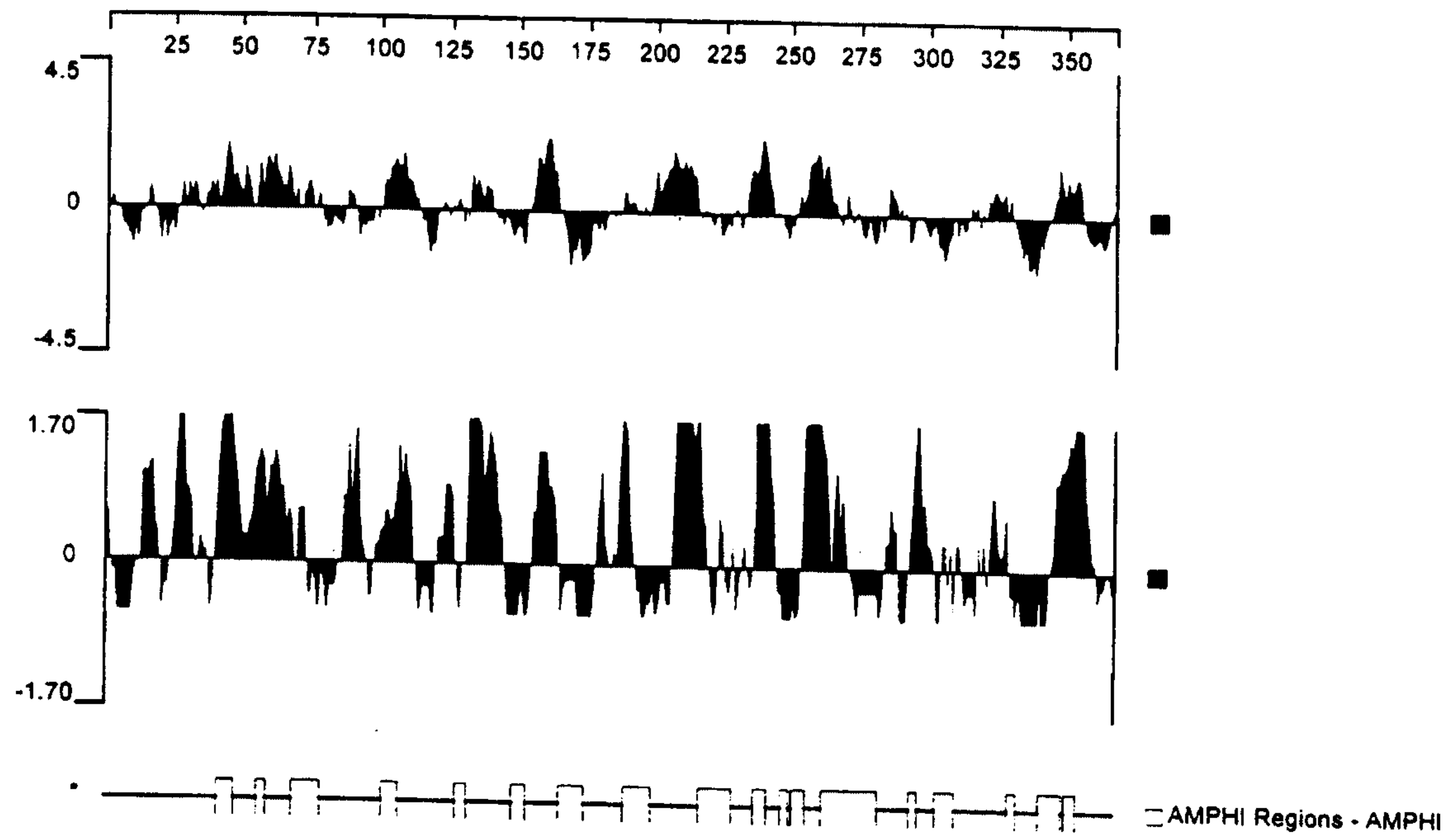


Fig. 14

128-1
Hydrophilicity Plot, Antigenic Index and AMPHI Regions

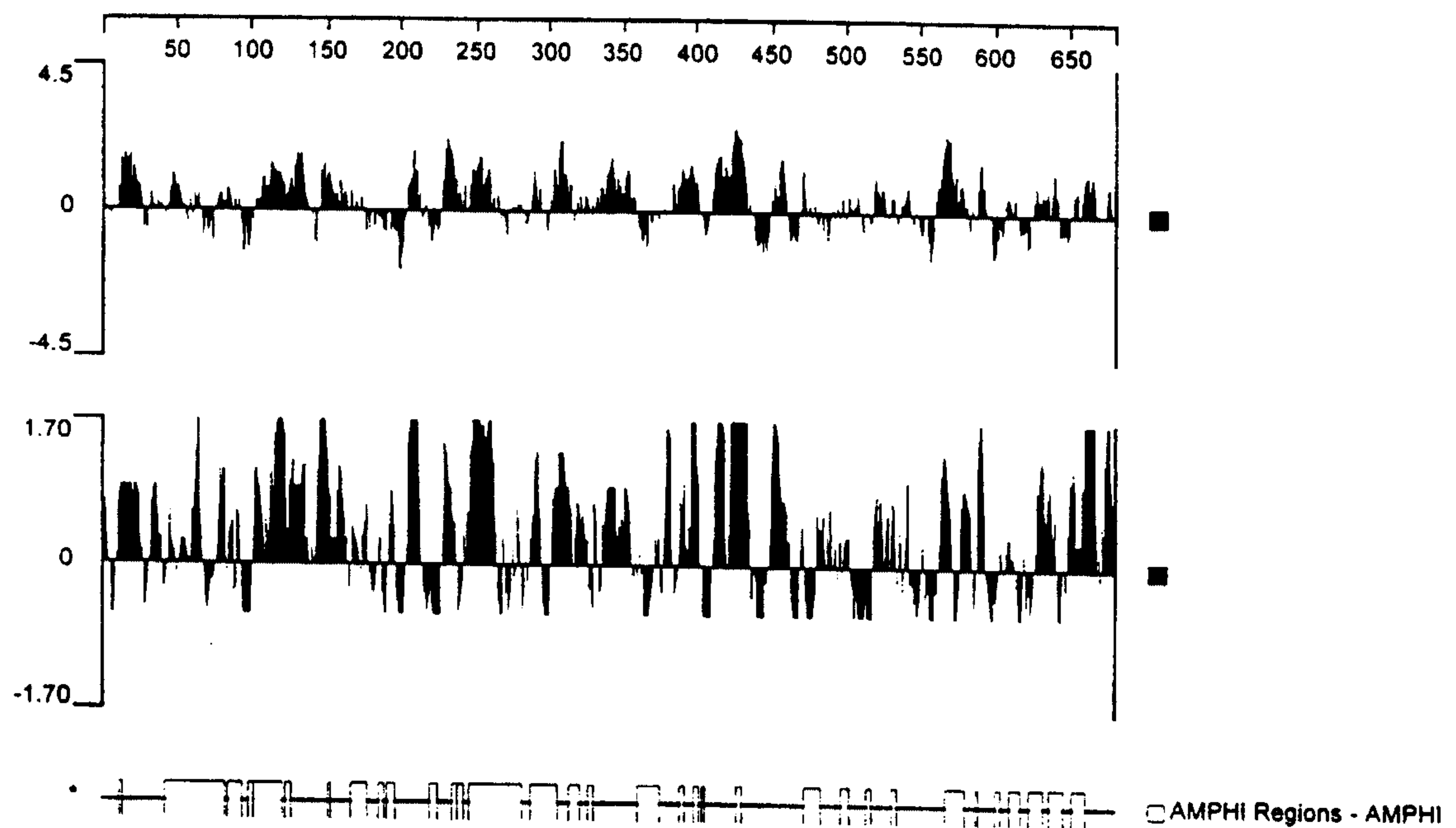


Fig. 15

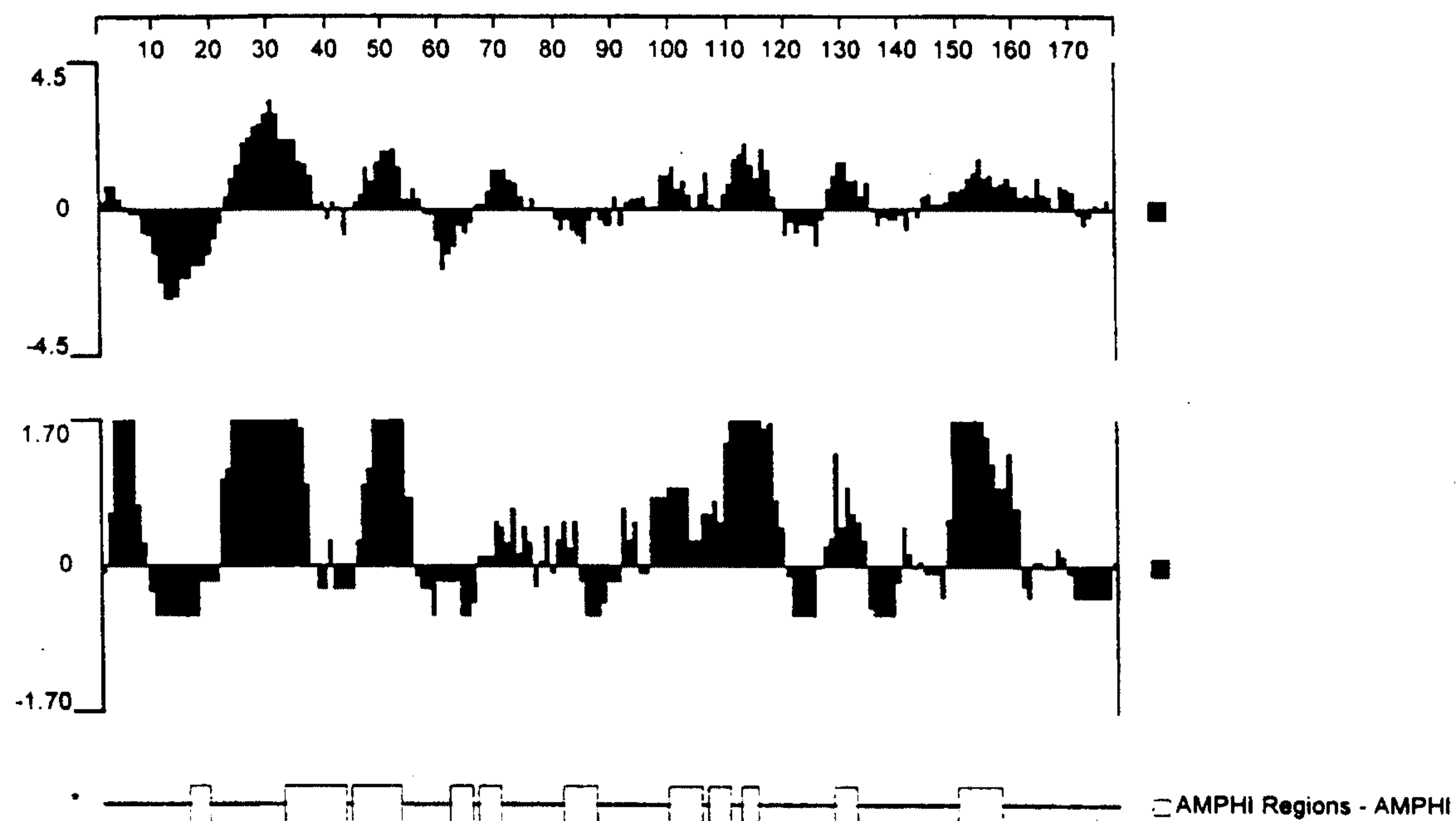
206Hydrophilicity Plot, Antigenic Index and AMPHI Regions

Fig. 16

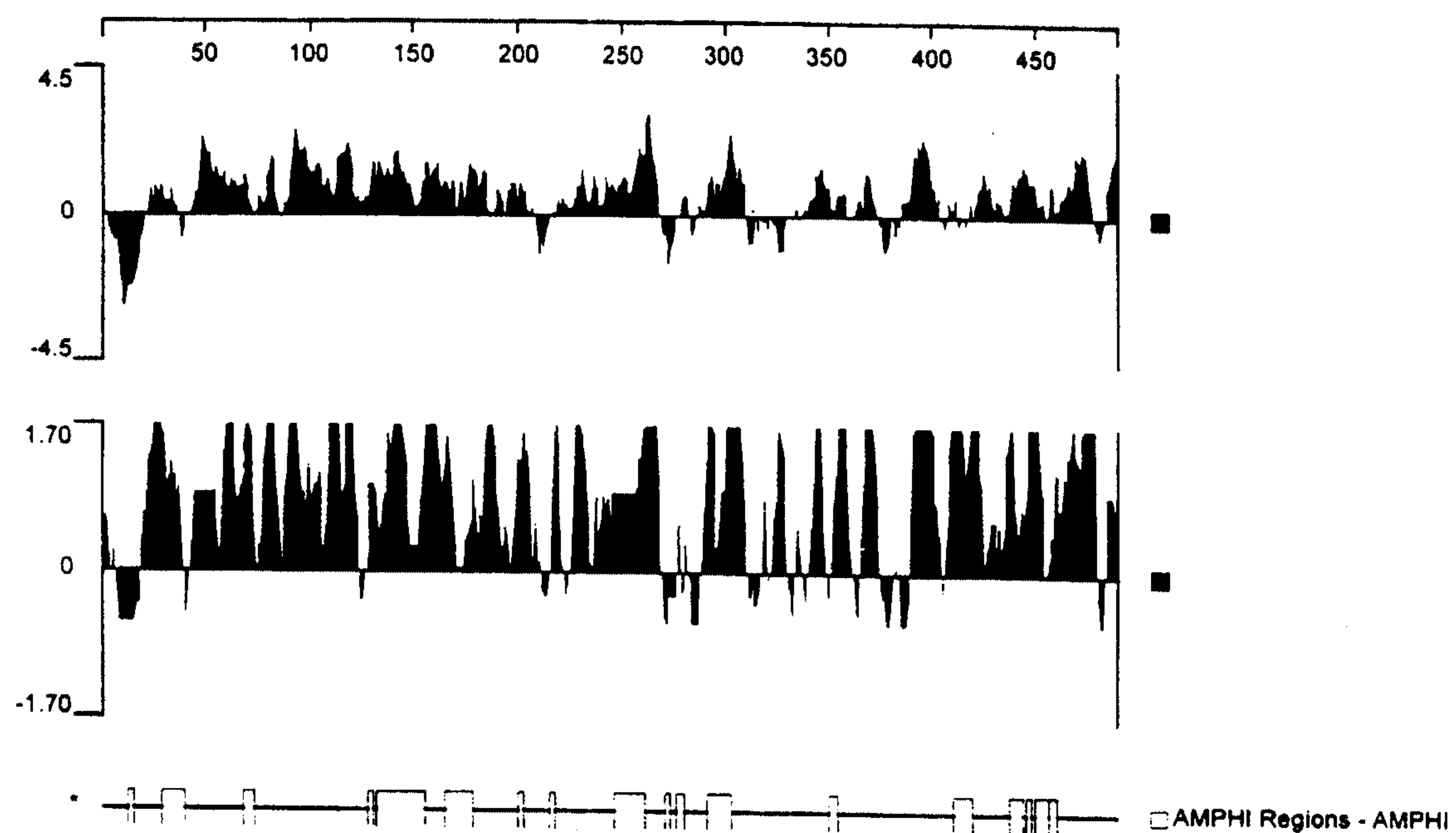
287Hydrophilicity Plot, Antigenic Index and AMPHI Regions

Fig. 17

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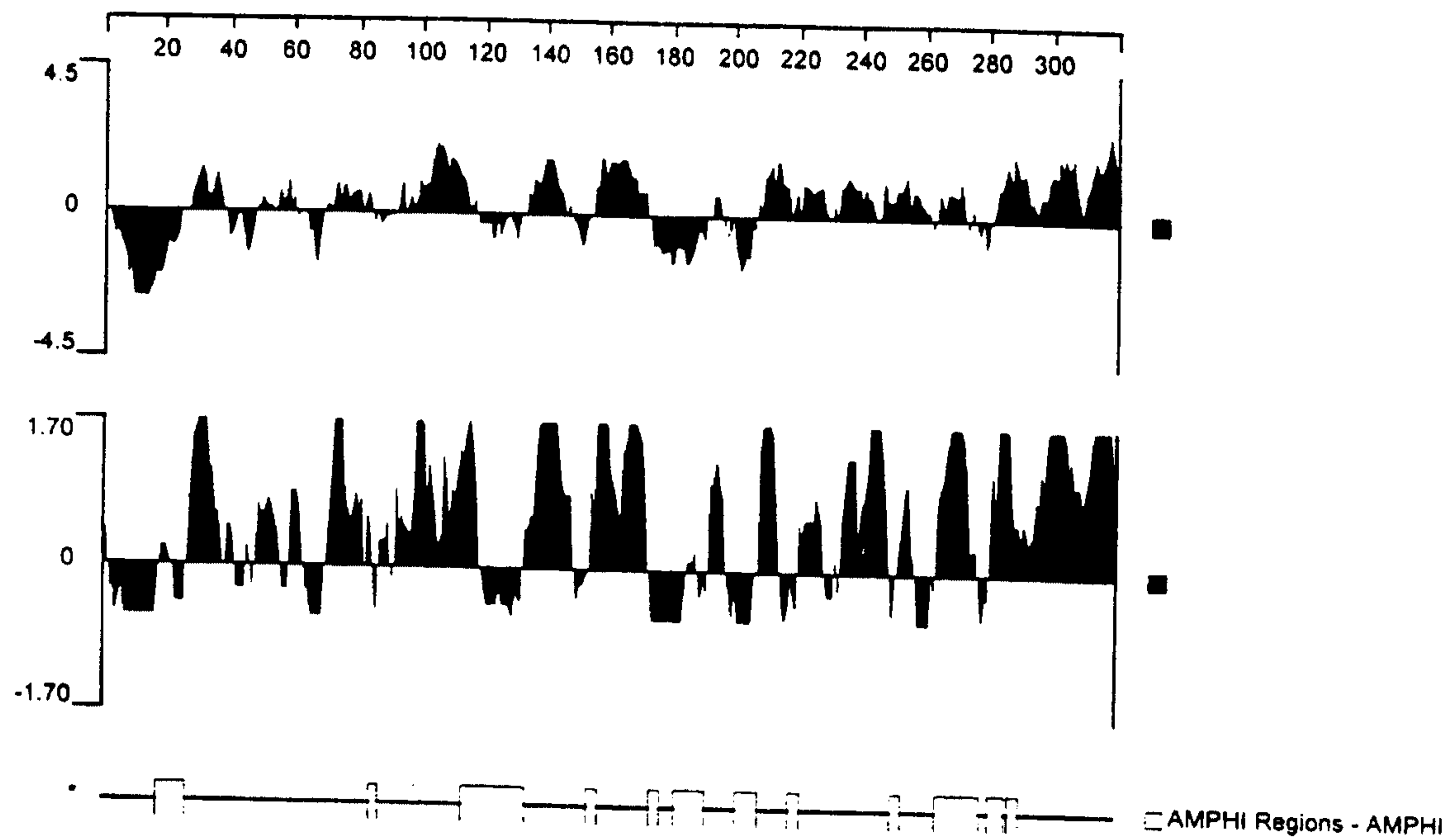
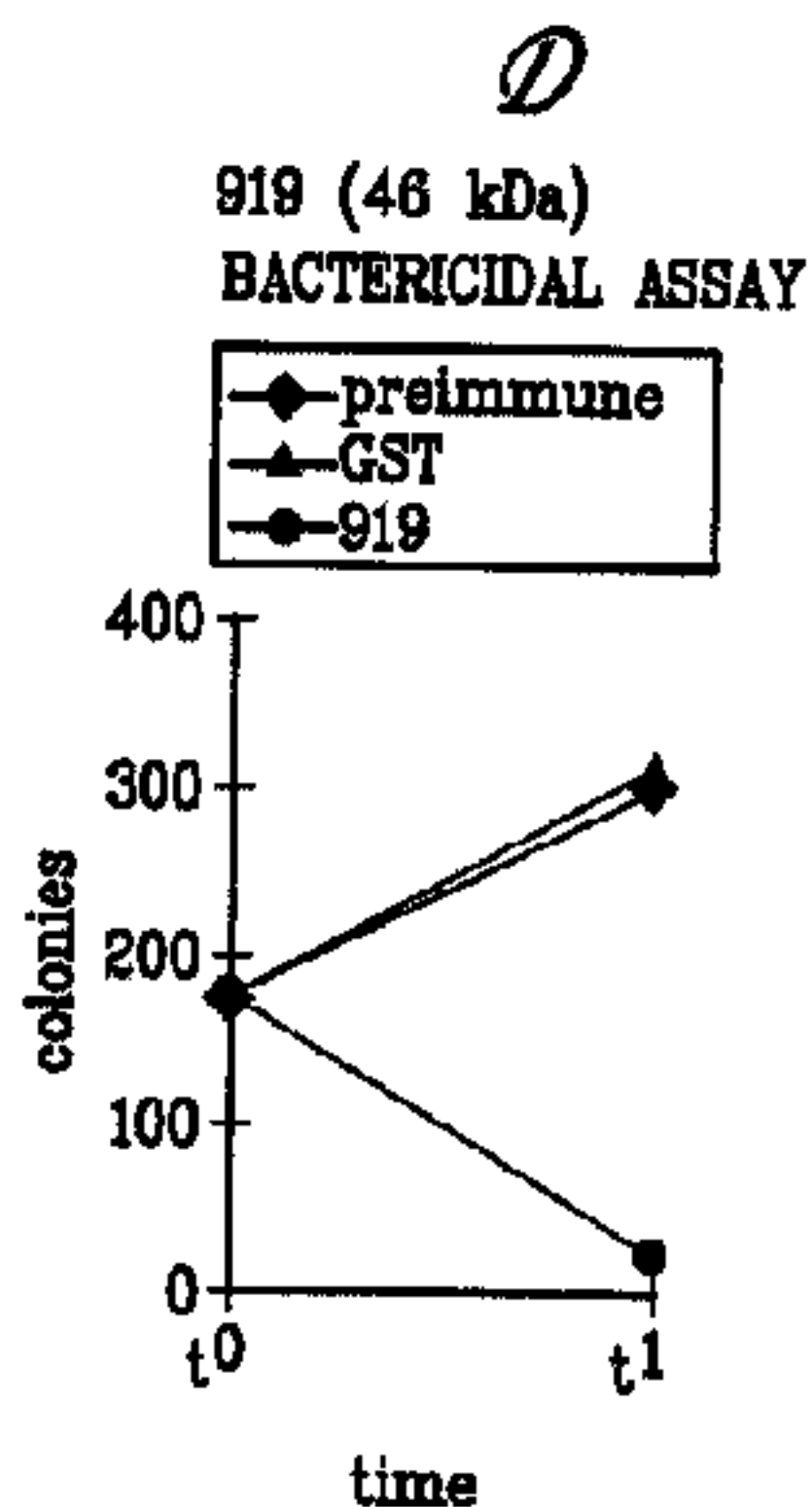
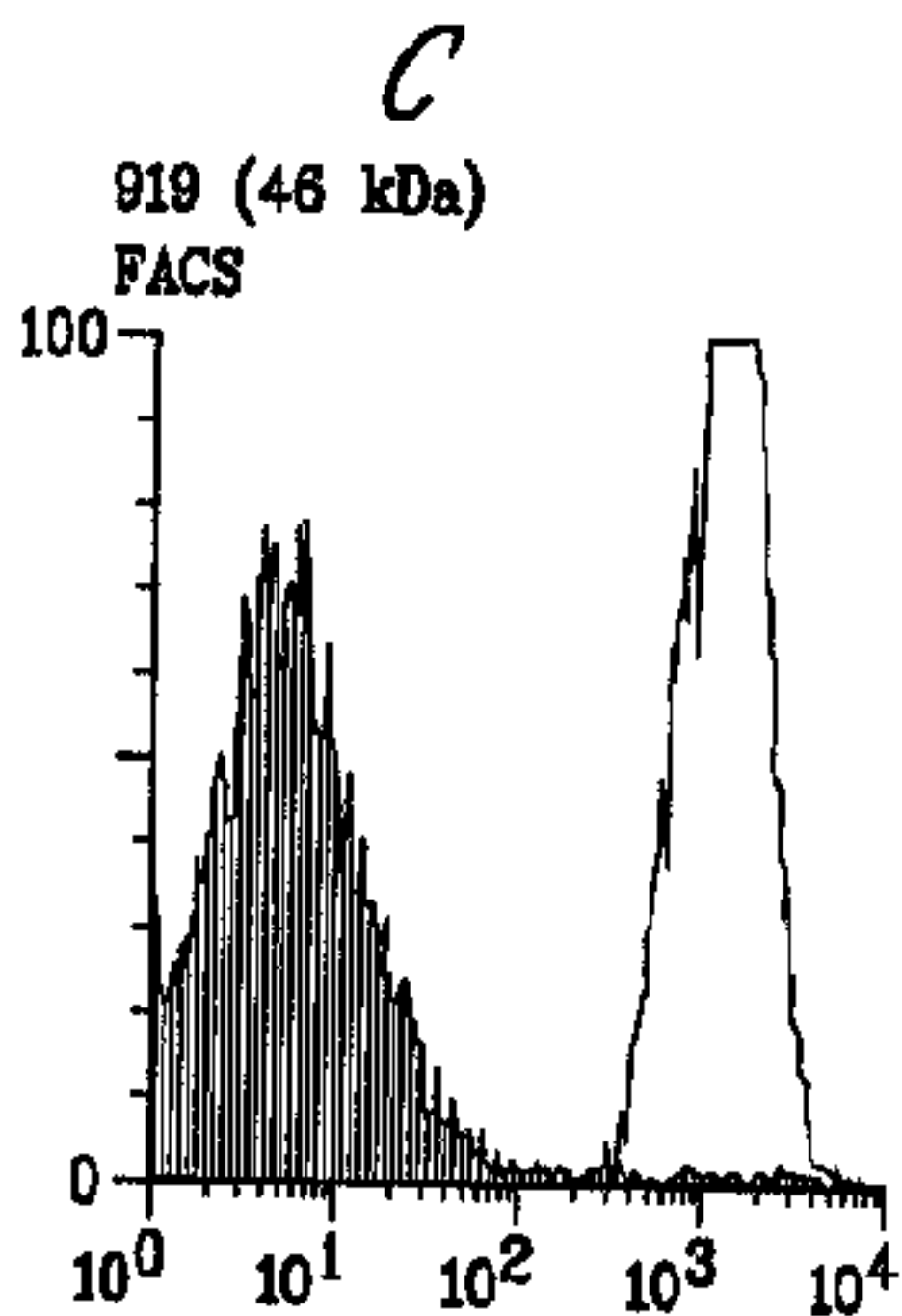
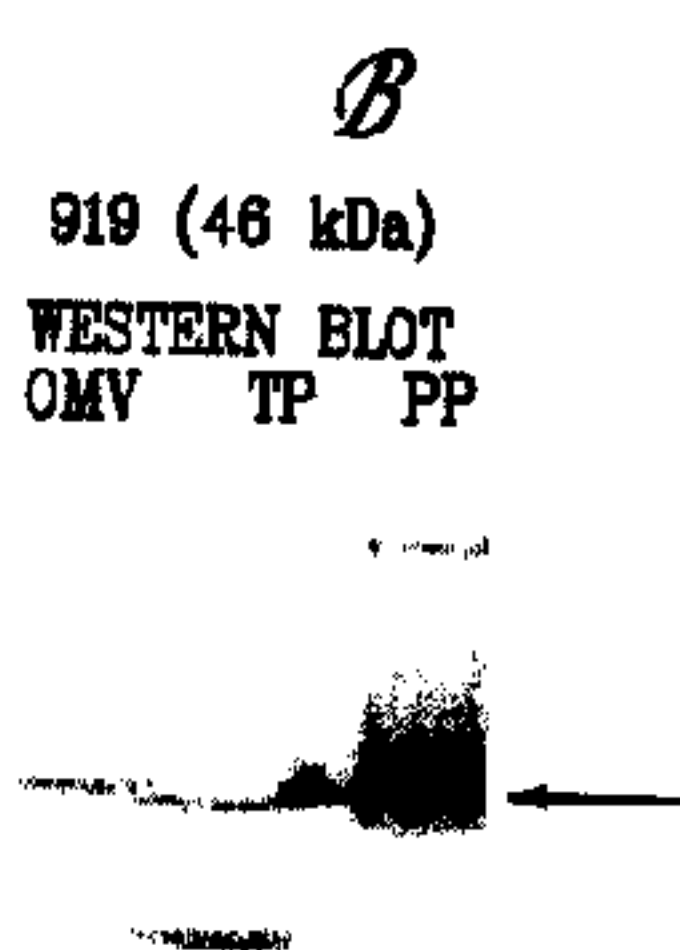
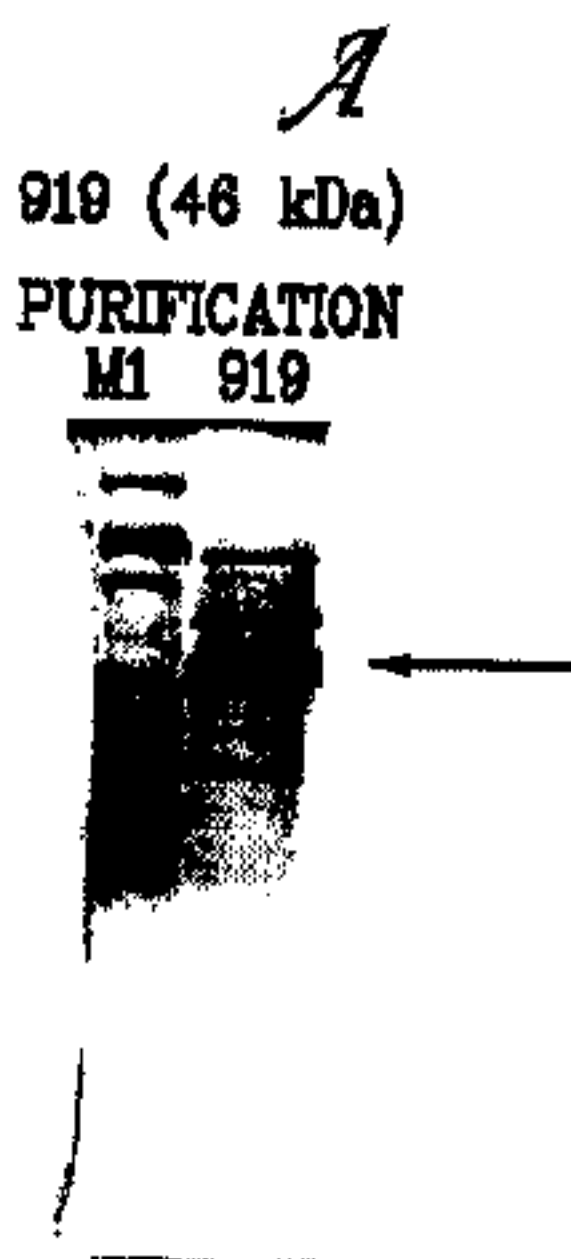
406Hydrophilicity Plot, Antigenic Index and AMPHI Regions

Fig. 18



E