HEWLETT® PACKARD

Routine 3.1 PVDF Sequencer Methods

Technical Note

HP G1005A
N-terminal Protein Sequencing System

TN 96-2

The N-terminal Routine 3.1 PVDF sequencer methods implement the Edman degradation chemistry (coupling, cleavage, and conversion) on Hewlett-Packard's column-based protein sequencer. These methods are appropriate for N-terminal amino acid sequence analysis of most proteins electroblotted to PVDF membrane in the nanomole to low picomole range. The methods are:

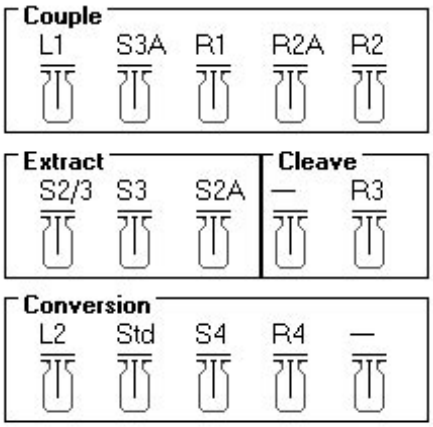
- **Routine column**
- **Routine converter**
- **Routine Cycle 1 (column)**
- **Routine PTH Std (converter)**
- **Column Prep (column)**

The Routine column and Routine converter methods typically control all sequence cycles with the exception of Cycle 1 for each sample. These methods are initiates by the Routine sequence program.

The method Routine Cycle 1 is used in the Routine sequence program as the column method for cycle 1. It provides an initial sample cleanup and performs a double coupling on the N-terminal amino acid. There is no specialized cycle 1 method for the converter.

The Routine PTH Std methods delivers 10 pmol/100 µl PTH-amino acid standard solution to the online HPLC allowing quantitation and identification of sequenced amino acids. You may choose to run a PTH amino acid standard using Routine PTH Std when scheduling your sample.

The Column Prep method is used to wash the sequencer column prior to sample loading. This is especially important for low-level (<10 pmol) samples.



The Routine 3.1 PVDF sequencer method requires the bottle configuration shown. These reagents and solvents are purchased from Hewlett-Packard (HP reagent/solvent kit G2046A). These methods produce less than 1mL/cycle of liquid waste.

L1	**neat acetonitrile**
S3A	**trifluoroaceticacid (TFA) in water (1:499)**
R1	**phenylisothiocyanate (PITC) in heptane (3:97)**
R2A	**octylamine in heptane (3:97)**
R2	**diisopropylethylamine (DIEA) in 1-propanol/water (1:3:6)**
S2/3	**acetonitrile/toluene (23:77)**
S3	**acetonitrile/toluene (15:85)**
S2A	**neat ethyl acetate**
R3	**neat trifluoroacetic acid (TFA)**
L2	**neat methanol**
Std	**mixture of PTH amino acids (10 pmol/100 µl) in acetonitrile with DPTU as marker in water (1:3)**
S4	**acetonitrile/water (10:90)**
R4	**trifluoroacteic acid (TFA) in water (1:3)**

Method details

The Steps in the Routine methods are described below. All sequencer methods are accessed by choosing the top menu item Edit/Method in the Protein Sequencer window. The steps indicate the ranges in volume that are appropriate for the various reagent/solvent deliveries. The sequencer column configuration used with Routine 3.1 PVDF method consists of a membrane-compatible empty column on top mated to a strong anion exchange (SAX) sample column on bottom.

Routine 3.1 PVDF column method
Total time: 45.4 min (2722.1 sec)

Step 2: The delivery of R2A should completely fill the column (both halves).

Steps 5, 8: The delivery of R1 should completely fill the column (both halves).

Step 11: The volume of R2 delivered during Step 11 should completely saturate the PVDF membrane(s).

Step 12: The membrane(s) should still be moist at the end of this step following removal of excess R2 and remain moist during the coupling reaction.

Step 15: The membrane(s) should be completely dry after this step.

Steps 18, 20, 22, 24: These solvent deliveries completely fill both the membrane-compatible and the SAX column, followed by drying.

Step 25: The membrane(s) should be dry after this step.

Step 28: No more than the upper frit of the membrane-compatible column should be wet.

Step 31: The volume of S4 delivered to the converter should be 50 µl (±5 µl).

Steps 33, 37, 41: The PVDF column and the SAX column should be completely filled with solvent during this step. Solvent should not enter the RV6 valve before the flow is directed to the converter.

Steps 34, 38: Membrane(s) should be saturated with solvent during these steps.

Step 44: Membrane(s) should be dry after this step.

Method (Column): Routine 3.1
PVDF Cycle 1
Total time: 61.2 min (3670.1 sec)

Steps 2, 4, 6: Cycle 1 begins with S3A washes to remove contaminants from the PVDF sample. During these steps, the columns should be completely filled with liquid.

Step 9: The membrane(s) should be completely dry at the end of this step.

Step 11: The delivery of R2A should completely fill the column (both halves).

Steps 14, 17, 25: The delivery of R1 should completely fill the column (both halves).

Steps 20, 28: The volume of R2 delivered during Steps 20 and 28 should completely saturate the PVDF membrane(s).

Step 21, 29: The membrane(s) should still be moist at the end of these steps following removal of excess R2 and remain moist during the coupling reaction.

Steps 23, 32: The membrane(s) should be completely dry after these steps.

Steps 35, 37, 39, 41: These solvent deliveries completely fill both the PVDf and the SAX columns, followed by drying.

Step 42: The membrane(s) should be dry after this step.

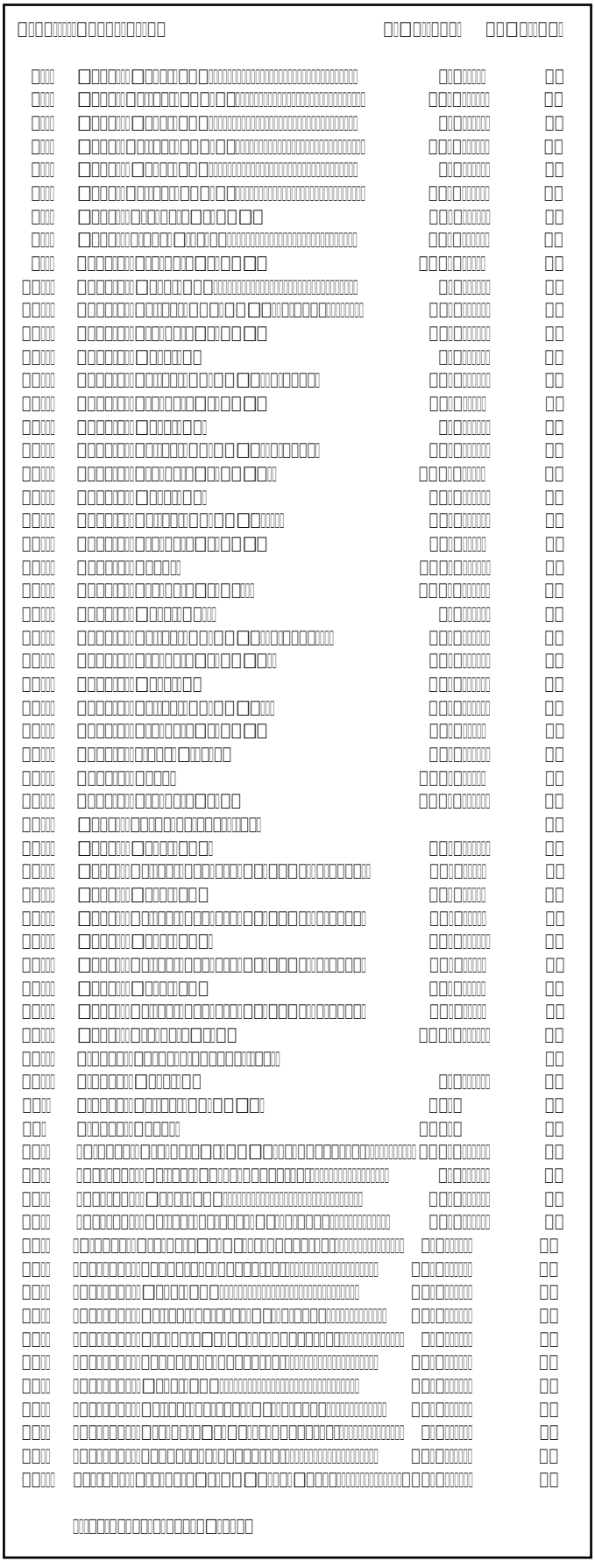
Step 45: No more than the upper frit of the membrane-compatible column should be wet.

Step 48: The volume of S4 delivered to the converter should be 50 µl (±5 µl).

Steps 50, 54, 58: The PVDF column and the SAX column should be completely filled with solvent during this step. Solvent should not enter the RV6 valve before the flow is directed to the converter.

Steps 51, 55: Membrane(s) should be saturated with solvent during these steps.

Step 61: Membrane(s) should be dry after this step.



Method (converter) Routine 3.1
PVDF
Total time: 33.1min (1988.1sec)

Step 1: During Step 1, the liquid in the converter partially evaporates but must not dry down completely (10-20 µl remains).

Step 2: During Step 2, the volume of R4 delivered to the converter should be approximately 70 µl (±5 µl).

TEST Delivery protocol:
To check the delivery volume of R4, copy Step 2 to the clipboard and run the clipboard. At the end of Step 2, remove the conversion flask from the heating chamber. Carefully unscrew the flask and measure the delivered volume.

Step 4: By the end of Step 4, the converter will have been dry for 100-200 secs.

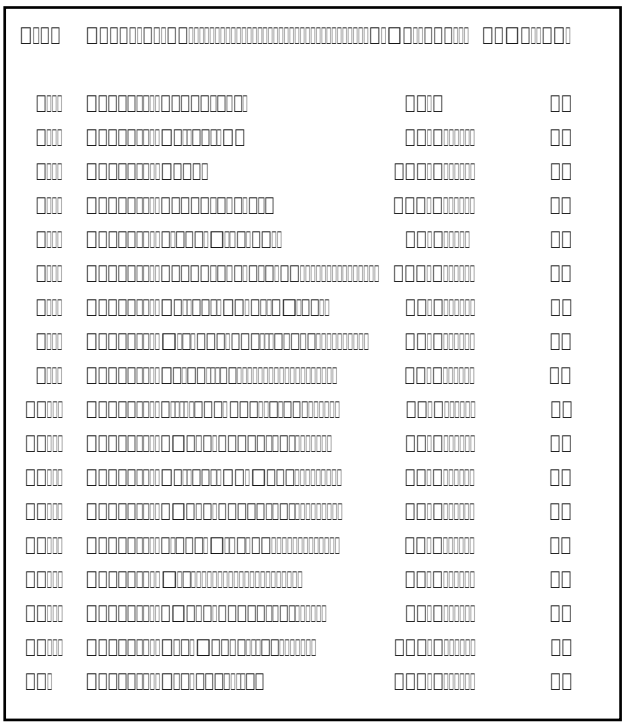
Step 5: By the end of Step 5, 100-120 µl of L2 will have been delivered to the converter.

Step 6: By the end of Step 6, the converter will have been dry for 75-100 secs.

Step 7: After Step 7, the volume of S4 delivered to the converter should be 75-80 µl.

TEST Delivery protocol:
To check the delivery volume of S4, copy Steps 7, 8, and 9 to the clipboard and run from the clipboard. At the end of Step 9, remove the conversion flask from the heating chamber. Carefully unscrew the flask and measure the delivered volume.

Step 10: Before injection the injector loop should be filled, leaving solvent visible in both the inlet and outlet lines of the injector loop.



Method (Converter): Routine 3.1/3.5 PTH Std
Total time: 34.3 min (2060.0 sec)

This Routine PTH-standard method is appropriate with any of the sequence programs: 3.1, 3.1 PVDF, or 3.5. Ten (10) pmols of PTH-amino acid standard are delivered to the conversion flask in this method.

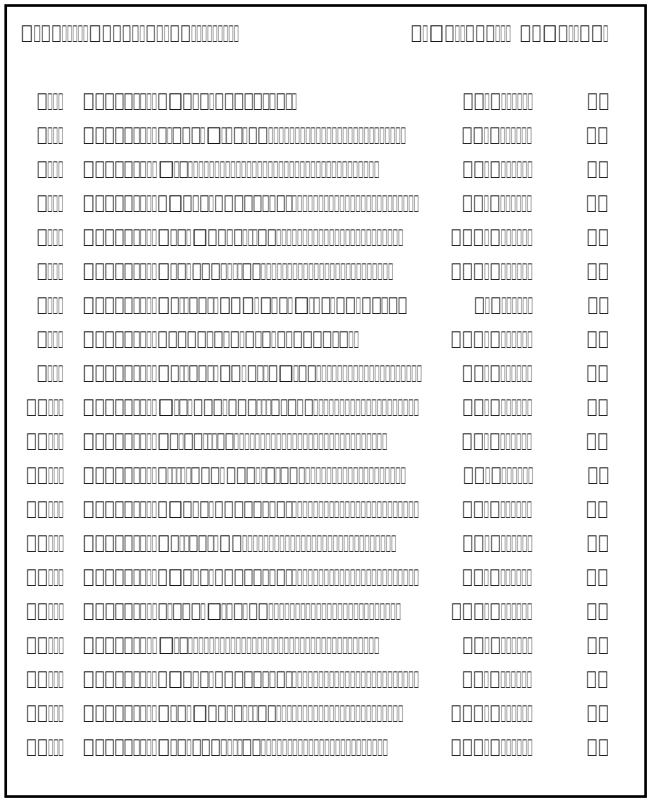
Step 7: Step 7 should add 100 µl (± 2 µl) of PTH-amino acid standard to the dry converter.

TEST Delivery protocol:
To check the delivery volume of PTH-standard: first remove the conversion flask from the heating chamber and allow to cool. Confirm that the PTH delivery time in the Test method is the same as the Routine PTH method delivery time. Run from the Clipboard the "Test 3.1/3.5 PTH Delivery" method. At Step 2 of the test method, carefully unscrew the flask and measure the delivered volume.

Step 8: The solvent is completely evaporated by end of the 180 sec dry.

Step 9: After Step 9, the volume of S4 delivered to the converter should be 75-80 µl.

Step 12: Before injection the injector loop should be filled, leaving solvent visible in both the inlet and outlet lines of the injector loop.



Method (Column): Column Prep 3.1 PVDF

Total time:20.5 min (1232.3 sec)

Steps 3, 14, 20, 23, 26, 28, 30, 32: These solvents deliveries completely fill the column, followed by drying.

Steps 6, 17: These reagents deliveries should completely fill the column (both halves).

Steps 9: The delivery of R2 in Step 9 should completely fill the lower SAX column.

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