International Bitterness Units

DOC316.53.01519

Iso-Octane Method **Method 10288**

2 to 300 IBU **TNTplus® 817**

Scope and application: For beer and wort.

Test preparation

Items to collect

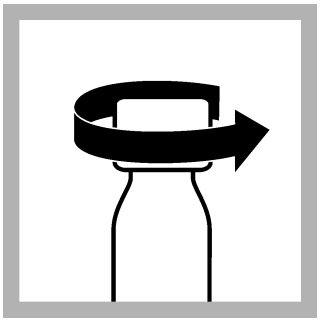
Description	Quantity
1-cm quartz sample cell	1
Pasteur pipet (150 mm) with pipet filler	1
IBU TNTplus® Reagent Set	1

Refer to Consumables and replacement items on page 4 for order information.

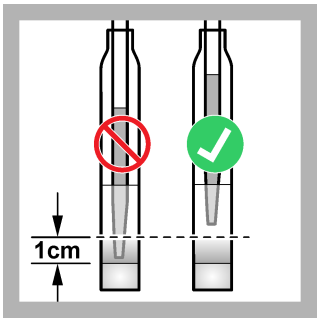
Before starting

Review the safety information and the expiration date on the package.
Use only a DR 5000 or DR 6000 for this test.
Use only 1-cm quartz sample cells for this test.
Fully clean the quartz sample cell between reagent sets. Clean the sample cell with alcohol (isopropyl or pure ethanol), rinse with water, clean with more alcohol and then let air dry.
Use only a glass pipet and pipet filler for this test. Keep the iso-octane phase out of the pipet filler. UV-active substances can cause inconclusive results (e.g., plasticisers).
Set a zero value on the spectrophotometer for each TNTplus® reagent set. Changes in the iso-octane quality of the reagent have a direct effect on the measurement result.
Use the same quartz sample cell to set the zero value and to measure the samples. Sample cell differences have a direct effect on the measurement result.
Degas samples that contain carbon dioxide before the test. For example, stir samples with a magnetic stirrer.
Do not filter the sample. The foam contains bitter substances.
Remove the wort and cloudy beer with a centrifuge before the test.
Review the Safety Data Sheets (MSDS/SDS) for the chemicals that are used. Use the recommended personal protective equipment.
Dispose of reacted solutions according to local, state and federal regulations. Refer to the Safety Data Sheets for disposal information for unused reagents. Refer to the environmental, health and safety staff for your facility and/or local regulatory agencies for further disposal information.

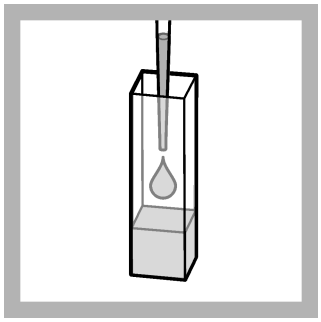
Test procedure



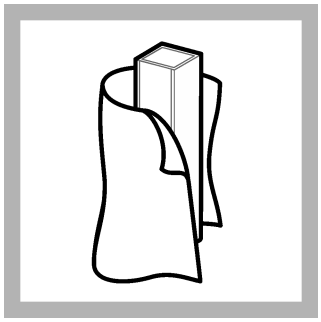
1. Remove the cap from the TNTplus® vial.



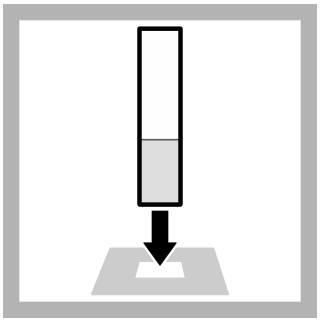
2. Prepare the blank: Remove most of the iso-octane phase (top layer) from the vial with a pasteur pipet. Keep a minimum of 1 cm of the iso-octane phase in the vial.



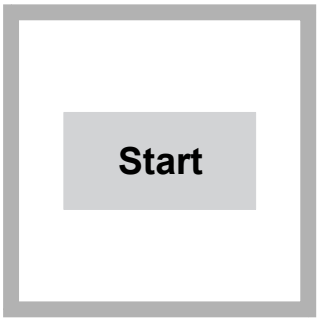
3. Put the iso-octane phase in a clean 1-cm quartz sample cell.



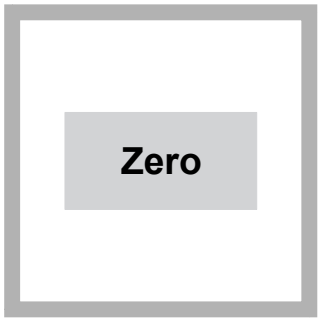
4. Clean the sample cell.



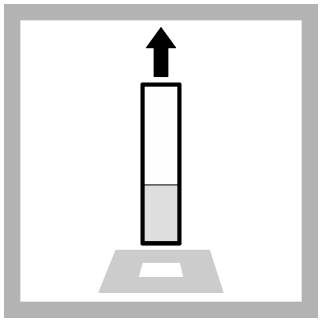
5. Insert the sample cell into the cell holder.



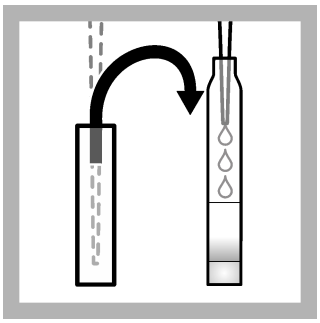
6. DR 5000/DR 6000: Go to Stored programs. Select program **817**.



7. Push **ZERO**.

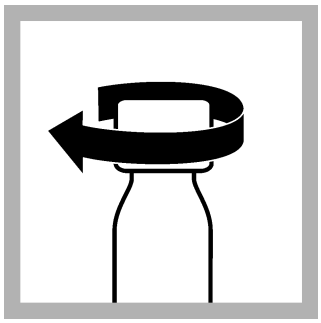


8. Remove the sample cell from the cell holder.

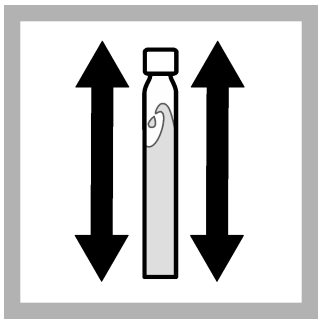


9. Remove all of the iso-octane phase from the sample cell with a pasteur pipet. Put the iso-octane phase in the TNTplus® vial.

10. Prepare the sample: Add 1 mL of sample slowly along the length of the vial inner wall with a pipet.

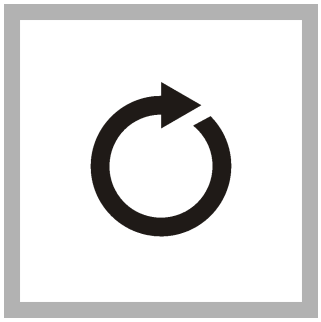


11. Put the cap on the vial.



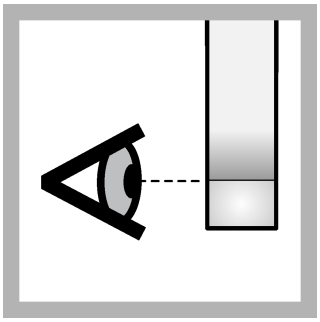
12. Shake the vial vigorously for 10 seconds.

13. Do not move the vial for 20 seconds.

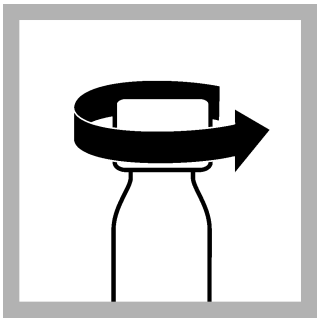


14. Do steps 12 and 13 two more times.

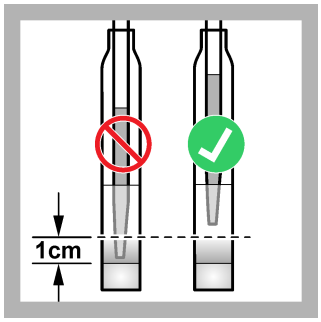
15. Do not move the vial for 1 minute.



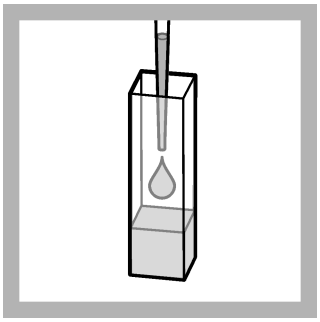
16. Make sure that the two phases (layers) are divided.



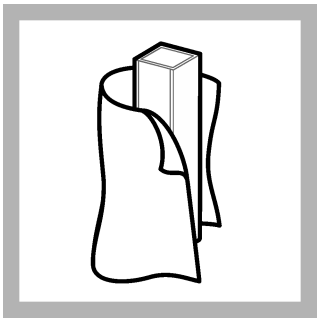
17. Remove the cap from the TNTplus® vial.



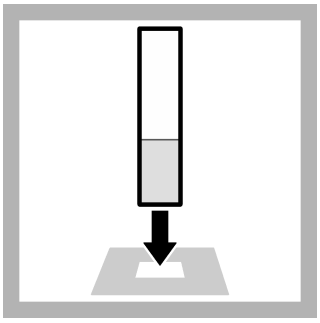
18. Remove most of the iso-octane phase (top layer) from the vial with a pasteur pipet. Keep a minimum of **1 cm** of the iso-octane phase in the vial.



19. Put the iso-octane phase in the 1-cm quartz sample cell.

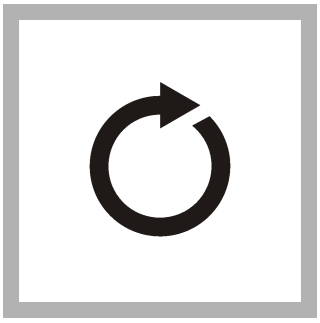


20. Clean the sample cell.



21. Insert the sample cell into the cell holder.

22. Push **READ**.



23. Do steps 10–22 with a new TNTplus® vial to measure the next sample.

For samples with largely different bitter units, clean the quartz sample cell between samples with iso-octane.

Summary of method

The bitter substances or recyclable materials in hops—primarily iso-α-acids—are extracted from the acidified sample with iso-octane. The concentration contained in the iso-octane extract is measured spectrophotometrically.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Item no.
IBU TNTplus® Reagent Set	1	25/pkg	TNT817

Required apparatus

Description	Quantity/test	Unit	Item no.
Sample cell, 1-cm quartz	1	each	2624410
Pipet filler	1	each	2676800
Pasteur pipet	1	each	Not sold by Hach

FOR TECHNICAL ASSISTANCE, PRICE INFORMATION AND ORDERING:
In the U.S.A. – **Call toll-free 800-227-4224**
Outside the U.S.A. – **Contact the HACH office or distributor serving you.**
On the Worldwide Web – **www.hach.com**; E-mail – **techhelp@hach.com**

HACH COMPANY
WORLD HEADQUARTERS
Telephone: (970) 669-3050
FAX: (970) 669-2932

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11/2016, Edition 1