Oxygen Demand, Biochemical

DOC316.53.01242

Dilution Method[1]

Method 10230

Scope and Application: For water and wastewater.

LBOD Measurement

[1] Adapted from *Standard Methods for the Examination of Water and Wastewater* and from Klein, R.L.; Gibbs, C. *Journal of Water Pollution Control Federation*, 1979, 51(9), 2257. USEPA recommended for compliance monitoring. Meets ASTM 888-05 (C)

Test preparation

Before starting the test:

The BOD test is a 5-day test. Follow all steps carefully to make sure that the test does not have to be repeated. The IntelliCAL™ LBOD probe measures dissolved oxygen in a 300 mL BOD bottle.
The dilution water for this test must not have an oxygen demand or any toxins. When incubated for 5 days at 20 °C, the dissolved oxygen concentration in the dilution water must not change by more than 0.2 mg/L.
Carbonaceous BOD (CBOD) can be determined by the addition of nitrification inhibitor. A test for CBOD is recommended for biologically treated effluents, samples seeded with biologically treated effluents and river water.

Collect the following items:

Description	Quantity
BOD bottles, 300-mL, glass, with glass stoppers and plastic caps	6
Dilution water containing nutrient buffer and seed (see *Dilution water preparation*)	varies
HQ40d or HQ30d meter with IntelliCAL LBOD101 probe	1
Nitrification inhibitor (for CBOD only)	1 bottle
Pipet, seriological	1
Incubator	1

See *Consumables and replacement items* for reorder information.

Dilution method

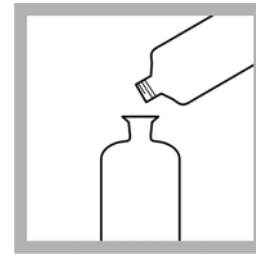
1. Prepare the dilution water using a BOD Nutrient Buffer Pillow. See *Dilution water preparation*.

2. Select the sample volumes. See *Sample size selection*.

3. Stir the sample gently with the pipet. Use the pipet to add the minimum sample volume to the first BOD bottle.

Add the remaining four sample volumes to four more BOD bottles.

When analyzing disinfected samples or industrial effluents, refer to *Interferences*.

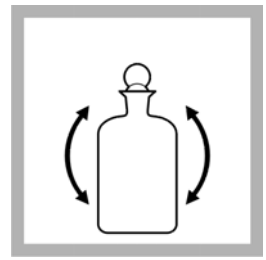


4. Fill an additional BOD bottle with dilution water only. This will be the dilution water blank.

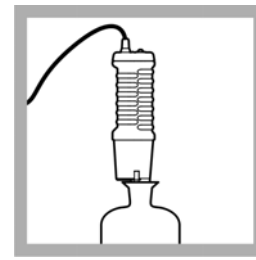
5. If the test is for CBOD, add two portions of Nitrification Inhibitor (approximately 0.16 g) to each bottle.

The oxidation of nitrogen compounds will be prevented. Report results as CBOD.

6. Fill each bottle to just below the lip with dilution water. Allow the dilution water to flow down the sides of the bottle to prevent air bubbles from becoming trapped in the bottle.



7. Stopper the bottles carefully to prevent air bubbles from becoming trapped. Press down on the stopper and invert the bottles several times to mix.



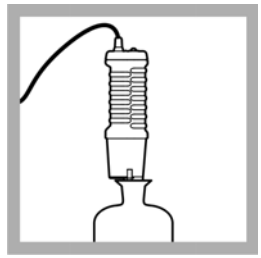
8. Probe calibration is required before initial and final BOD readings. Refer to the *Calibration* section of this procedure.

Be sure to measure the DO of the blank.

An initial DO measurement is not necessary when the graphical method (not for reporting) is used for calculation.

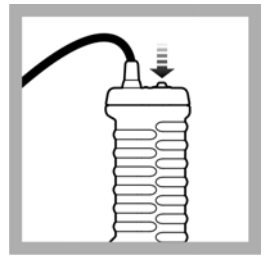
Dilution method (continued)

9. Rinse the LBOD probe with deionized water.

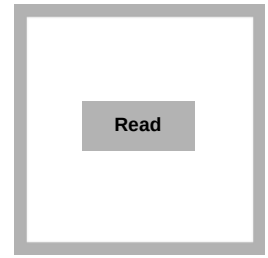


10. Place the LBOD Probe in the BOD Bottle containing the sample.

Make sure there are no air bubbles trapped under the probe.



11. Engage the stir paddle on the LBOD probe by pushing the button on the top of the probe. The green indicator light on the top of the probe will illuminate when the stirrer is running.



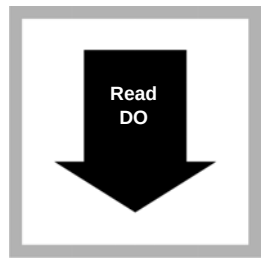
12. Press the key under **READ**. After the measurement has stabilized, the dissolved oxygen value will show on the display.

13. Record the value.

Data is stored automatically in the Data Log when Press to Read or Interval is selected in the Setup Measurement Mode. When Continuous is selected, data will only be stored when the **GREEN/RIGHT** key under Store is pressed.

14. Remove the LBOD Probe from the bottle and stopper the bottles carefully to prevent air bubbles from becoming trapped. Add dilution water to the lip of each BOD Bottle to make a water seal.

Repeat Steps 9–14 for each BOD bottle.



15. After five days, measure the remaining dissolved oxygen concentration in each bottle.

At least 1.0 mg/L DO should be left in each BOD bottle.

16. Calculate the BOD value (see *BOD calculation—standard methods* or *BOD calculation—graphical method*).

Calibration

Water-saturated air calibration

1. Fill a BOD bottle ¾ full with water (225 mL). If a 0% calibration point is required, refer to the *Sulfite correction* section.
2. Put the BOD stopper in the BOD bottle and shake vigorously for about one minute to saturate the air with water.

3. Remove the stopper. Put the Intellical LBOD Probe into the BOD bottle for several minutes to reach equilibrium. Inspect the LBOD probe sensor cap surface to make sure it is dry. If the sensor cap is wet, carefully dry the cap with a non-abrasive cloth.
4. Make sure the meter is in the measurement screen. Press **CALIBRATION**.

   Note: *For the HQ40d meter with two probes connected, make sure the meter is in the single scree LDO101 mode.*

5. Press **READ**. When the measurement has stabilized, the calibrated measurement will show on the screen. The standard value will be highlighted.
6. Press **DONE** to view the calibration summary. The slope value is the comparison between the latest calibration and the factory calibration expressed as a percentage.

   Note: *If the calibration slope does not meet the acceptance criteria, the display will show "Slope out of range". Let the probe stand in water-saturated air for several minutes. When the probe reaches equilibrium, press* ***READ****.*

7. Press **STORE** to accept the calibration and return to the measurement mode. The calibration record is stored in the data log.

Note: *A successful calibration will show "OK" in the measurement screen.*

Sulfite correction

1. Fill a BOD bottle full with deionized water.
2. Add 300 mg of sodium sulfite to the bottle.
3. Add a small crystal of cobalt chloride.
4. Put the stopper in the BOD bottle and invert several times to mix the chemicals.
5. Put the LBOD probe in the bottle and engage the stirrer. This will help speed up the calibration. When the meter reaches a stable reading, press the calibration button on the meter.
6. After the 0% saturated message is displayed press **STORE**. After using sulfite, be sure to clean the probe thoroughly.
7. To clean the sulfite off of the probe, put the LBOD probe in a BOD bottle full of water, activate the stirrer and run for 10 minutes to remove sulfite residue.

Dilution water preparation

The dilution water must be prepared very carefully to make sure that no source of oxygen demand or toxins are added. The water that is used to prepare the dilution water must be of very high quality. The water must not have any organic compounds or any toxic compounds such as chlorine, copper and mercury.

Use the following guidelines to make sure the dilution water is of high quality.

Guidelines

- Use distilled water from an alkaline permanganate distillation for the best results.
- Do not use deionized water from ion exchange columns. The resins in the cartridges (especially new cartridges) will occasionally release organic materials that have an oxygen demand. In addition, bacteria can grow on the columns and contaminate the dilution water.
- Store the distilled water in clean jugs in an incubator at 20 °C. Shake the jugs to saturate the water with air or cap the jugs loosely and store for 24 hours or more.

- A small aquarium pump or air compressor can be used to saturate the water with air. Make sure that the air is filtered and that the filter does not grow bacteria.
- Add the nutrients and seed (if necessary) to the distilled water immediately before the test.
- The dissolved oxygen concentration in the dilution water must not change by more than 0.2 mg/L when incubated for 5 days at 20 °C.

Procedure

1. Prepare and store the distilled water at 20 °C (see *Guidelines*).
2. Select a BOD nutrient buffer pillow from the *BOD nutrient buffer pillows* table.
3. Shake the pillow to mix the contents.
4. Add the contents of the pillow to the distilled water. Cap the jug and shake vigorously for one minute to dissolve the nutrients and to saturate the water with air.
5. If the sample is known to be low in bacteria, for example industrial waste or sewage that has been disinfected, add 3 mL of bacterial seed to each liter of the dilution water. Use raw sewage for the bacterial seed. Allow the sewage to stand undisturbed at 20 °C for 24 to 36 hours before use. Pipet from the upper portion of the sewage. Make sure to measure the BOD of the seed so that it can be subtracted from the BOD of the sample.

Table 467 BOD nutrient buffer pillows

Volume of dilution water to prepare	BOD nutrient buffer pillow catalog no.
300 mL (add pillow to each BOD bottle)	1416066
3 liters	1486166
4 liters	2436466
6 liters	1486266
19 liters	1486398

Note: *To prepare dilution water by the conventional method, pipet 1 mL of each of the following solutions per liter of distilled water at 20 °C: Calcium Chloride Solution, Ferric Chloride Solution, Magnesium Sulfate Solution and Phosphate Buffer Solution. Cap the bottle and shake vigorously for one minute. The Phosphate Buffer Solution should be refrigerated to decrease the rate of biological growth. Use care with all solutions to avoid contamination.*

Sample size selection

Make an estimation of the sample volumes that are necessary for the test. At least 2.0 mg/L of dissolved oxygen (DO) should be consumed during the test and at least 1.0 mg/L DO should be left in the BOD bottle.

Samples such as raw sewage will have a high BOD. Small sample volumes must be used because large samples will consume all of the oxygen. Samples with a low BOD must use larger sample volumes to make sure that enough oxygen is consumed to give accurate results.

The elevation of the laboratory changes the amount of oxygen that can dissolve in water (refer to the *Oxygen values at various altitudes (20 °C)* table). At higher elevations, the amount of oxygen that can dissolve in water decreases, so less oxygen is available to microorganisms.

Procedure

1. Refer to the *Minimum sample volume* table to select the minimum sample volume. For example, if a sewage sample is estimated to contain 300 mg/L BOD, the minimum sample volume is 2 mL. For sewage effluent with an estimated BOD of 40 mg/L, the minimum sample volume is 15 mL.

2. Refer to the *Maximum sample volume* table to select the maximum sample volume. At 1000 feet, with an estimated BOD of 300 mg/L, the largest sample volume is 8 mL. For a BOD of 40 mg/L the maximum volume is 60 mL (also at 1000 feet).
3. Select three other sample volumes between the minimum and maximum volumes so that there are five sample volumes total.

Table 468 Minimum sample volume

Sample type	Estimated BOD (mg/L)	Minimum sample volume (mL)
Strong trade waste	600	1
Raw and settled sewage	300	2
	200	3
	150	4
	120	5
	100	6
	75	8
	60	10
Oxidized effluents	50	12
	40	15
	30	20
	20	30
	10	60
Polluted river waters	6	100
	4	200
	2	300

Table 469 Maximum sample volume

BOD at sea level	BOD at 1000 ft	BOD at 5000 ft	Maximum sample volume (mL)
2460	2380	2032	1
1230	1189	1016	2
820	793	677	3
615	595	508	4
492	476	406	5
410	397	339	6
304	294	251	8
246	238	203	10
205	198	169	12
164	158	135	15
123	119	101	20
82	79	68	30
41	40	34	60
25	24	21	100
12	12	10	200
8	8	7	300

Table 470 Oxygen values at various altitudes (20 °C)

Altitude (ft)	Oxygen value (mg/L) in water saturated with air
Sea level	9.2
1000	8.9
2000	8.6
3000	8.2
4000	7.9
5000	7.6
6000	7.4

BOD calculation—standard methods

Use the Standard Methods calculation when the results must be reported to a regulatory agency.

When dilution water is not seeded:

$$BOD_5,\ mg/L = \frac{D_1 - D_2}{P}$$

When dilution water is seeded:

$$BOD_5,\ mg/L = \frac{(D_1 - D_2) - (B_1 - B_2)f}{P}$$

where:

BOD_5 = BOD value from the 5-day test

D1 = DO of diluted sample immediately after preparation, in mg/L

D2 = DO of diluted sample after 5 day incubation at 20 °C, in mg/L

P = Decimal volumetric fraction of sample used

B1 = DO of seed control before incubation, in mg/L

B2 = DO of seed control after incubation, in mg/L

f = ratio of seed in diluted sample to seed in seed control =

(% seed in diluted sample)/(% seed in seed control) OR

If seed material is added directly to sample or to seed control bottles:

f = (volume of seed in diluted sample)/(volume of seed in seed control)

Report results as $CBOD_5$ if nitrification inhibitor was added.

Averaged results are acceptable if more than one sample dilution meets all of the following criteria:

- The remaining DO is at least 1 mg/L
- The final DO value is at least 2 mg/L lower than the initial DO value
- There is no evidence of toxicity at higher sample concentrations
- There are no obvious anomalies

BOD calculation—graphical method

Important Note: *The Graphical Method cannot be used when the results must be reported to a regulatory agency.*

1. Plot the mg/L dissolved oxygen (DO) remaining in each diluted sample versus the mL sample taken. Draw the best straight line through the plotted points. Refer to *Dissolved Oxygen per mL of Sample*.

Note: *An erroneous point is visually evident at this time and can be disregarded. However, at least three points should be on the line or very close to it. For unseeded dilution water, the line should cross the mg/L oxygen remaining scale near or below the oxygen saturation value for the altitude of the laboratory as discussed in Dilution water preparation.*

2. To calculate the BOD, use the following equation which is mathematically equivalent to the BOD equation in Standard Methods.

   mg/L BOD = (A x 300) – B + C

   where:

   A = the slope

   The slope of the line is equal to the mg/L DO consumed per mL of sample taken. Take any point on the line and subtract the mg/L DO remaining at that point from the mg/L DO where the line crosses the DO scale (Y intercept, mg/L DO remaining). Divide the difference by the mL of sample at the point chosen.

   300 = the volume of the BOD bottle

   B = the Y intercept

   This is the DO value where the line crosses the “DO remaining” scale. (This should be very close to the actual dilution water blank value.)

   C = the sample DO

   This is the DO of the undiluted sample.

   Another way to write this equation is:

   mg/L BOD = (Slope x 300) – Y intercept + Sample DO

   Note: *If the best straight line is obtained by linear regression through use of a calculator, the sign (-) of the slope must be changed (+) before multiplying by 300.*

Example:
The mg/L DO remaining was determined for a series of four dilutions of domestic sewage after five days of incubation. Results were as follows:

mL of sample taken	mg/L DO remaining
2.0	7.50
3.0	6.75
6.0	4.50
9.0	2.25

The DO values were plotted versus the mL of sample taken and a straight line drawn as in *Dissolved Oxygen per mL of Sample*. If a set of BOD dilutions is run correctly with a homogeneous sample, a graph of the mg/L DO remaining versus the sample volume would result in a straight line. The value where the line intersects the y-axis is equal to the DO content of the dilution water after incubation, although this is not actually measured. In this case, it was equal to 9.0 mg/L and the DO of the domestic sewage sample was assumed to be zero. If another type of sample is used, the DO of an undiluted sample should be measured either by the Winkler titration or potentiometrically.

The American Public Health Association formula for calculating BOD also can be written as follows (not approved for reporting purposes):

$$\frac{\text{mg/L DO remaining w/smaller sample volume} - \text{mg/L DO remaining w/larger sample volume}}{\text{mL of larger sample volume} - \text{mL of smaller sample volume}} \times 300 - DO_D + S = \text{mg/L BOD}$$

Using this information in the example:

mg/L DO remaining with smaller sample volume = 7.50

mg/L DO remaining with larger sample volume = 2.25

mL of larger sample volume = 9.0

mL of smaller sample volume = 2.0

300 = volume (mL) of BOD bottle

DO_D = mg/L DO of dilution water = 9.0

S = mg/L DO of sample = assumed in this case to be zero

Therefore:

$$\frac{7.50 - 2.25}{9.0 - 2.0} \times 300 - 9 + 0 = \text{mg/L BOD} = 216 \text{ mg/L BOD}$$

Using the equation below:

(slope x 300) – Y-Intercept + sample DO = mg/L BOD

To determine slope, arbitrarily select point A in Figure 1. At this point the mg/L DO remaining is equal to 3.0 mg/L. The mL of sample at this point is 8 mL.The difference between the y-intercept of 9.0 mg/L and 3.0 mg/L equals 6 mg/L; 6 mg/L divided by 8 mL = 0.75 mg/L per mL.

slope = 0.75 mg/L per mL

Y intercept = 9.0 mg/L

sample DO = 0 (Because the sample is domestic sewage, this is assumed to be zero.)

Therefore:

(0.75 x 300) – 9.0 + 0 = mg/L BOD = 216 mg/L BOD

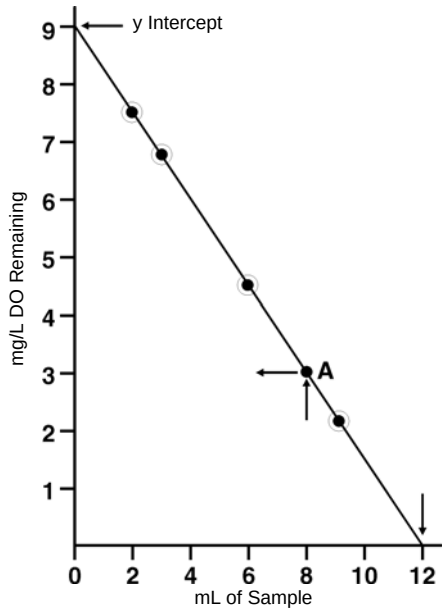


Figure 35 Dissolved Oxygen per mL of Sample

Interferences

Many chlorinated and industrial effluents require special handling to ensure reliable BOD results. Usually, careful experimentation with the particular sample will indicate what modifications should be made to the test procedure.

Toxins in the sample will adversely affect any microorganisms present and result in lower BODs.

To eliminate small amounts of residual chlorine, allow the sample to stand for one to two hours at room temperature. For larger quantities, determine the amount of sodium thiosulfate to add to the sample as follows:

c. Measure 100 mL of sample into a 250-mL Erlenmeyer flask. Using a 10-mL serological pipet and a pipet filler, add 10 mL of 0.020 N Sulfuric Acid Standard Solution and 10 mL of Potassium Iodide Solution, 100-g/L, to the flask.

d. Add three full droppers of Starch Indicator Solution and swirl to mix.

e. Fill a 25-mL buret with 0.025 N Sodium Thiosulfate Standard Solution and titrate the sample from dark blue to colorless.

f. Calculate the amount of 0.025 N Sodium Thiosulfate Standard Solution to add to the sample:

$$\text{mL 0.025 N sodium thiosulfate required} = \frac{\text{mL titrant used x volume of remaining sample}}{100}$$

g. Add the required amount of 0.025 N Sodium Thiosulfate Standard Solution to the sample. Mix thoroughly. Wait 10 to 20 minutes before running the BOD test.

To eliminate the effect of phenols, heavy metals or cyanide, dilute the sample with high quality distilled water. Alternately, the seed used in the dilution water may be acclimatized to tolerate such materials. Acclimatize seed as follows:

a. Fill a one-gallon stainless steel or plastic container with domestic sewage and aerate for 24 hours. Allow the heavier material to settle.

b. After settling for one hour, siphon off three quarts of material and discard.

c. Fill the container with a mixture of 90% sewage and 10% wastes containing the toxic material.

d. Aerate for 24 hours. Repeat steps b and c with increasing amounts of waste until the container holds 100% toxic waste material.

Optimum pH for the BOD test is between 6.5 and 7.5. Adjust samples to pH 7.2 with Phosphate Buffer Solution or 1 N Sulfuric Acid or Sodium Hydroxide Standard Solution if the pH is not in this range.

Cold samples may be supersaturated with oxygen and will have low BOD results. Fill a one-quart bottle about halfway with cold sample and shake vigorously for two minutes. Allow sample temperature to reach 20 °C before testing.

Accuracy check

Standard solution method
Required for accuracy check:

- BOD Standard Solution, Voluette® Ampule, 300-mg/L, 10-mL (300-mg/L of glucose and 300-mg/L of glutamic acid)
- Seeded dilution water
- 4 BOD bottles
- 1.0–4.0 mL Class A volumetric pipets
- TenSette Pipet

1. Open the standard solution ampule.
2. Use a pipet to add 1.00, 2.00, 3.00 and 4.00 mL of standard into four BOD bottles.
3. Fill the bottles with seeded dilution water and measure the DO concentration.
4. Incubate the bottles at 20 °C for five days.
5. Measure the DO remaining in each bottle.
6. Calculate the BOD value (refer to *BOD calculation—standard methods* or *BOD calculation—graphical method*).
7. Divide the value by two. The result for comparison with Standard Methods should be 198 (± 30.5) mg/L.

   Note: *The result must be divided by 2 to correspond with values reported in Standard Methods because the Standard Methods procedure uses 150 mg/L each of glucose and glutamic acid.*

Method performance

The following statements are true for dissolved oxygen when the measurement is below 10 mg/L DO and the temperature is kept between 10 and 30 °C for a single probe.

Instrument	Standard	Precision 95% Confidence Limits of Distribution
LBOD101	7.94–8.06 mg/L DO	7.97–8.03 mg/L DO

Summary of method

Biochemical Oxygen Demand (BOD) is a measurement of the oxygen requirements of municipal and industrial wastewaters and sewage. The test results are used to calculate the effect of waste discharges on the oxygen resources of the receiving waters. The BOD test is of limited value in measuring the actual oxygen demand because temperature change, biological population, water movement, sunlight, oxygen concentration and other environmental factors cannot be reproduced accurately in the laboratory. The BOD test is of greatest value after patterns of oxygen uptake for a specific effluent and receiving water have been established.

The BOD test is performed by incubating a sealed wastewater sample (or a prepared dilution) for the standard five-day period and then determining the change in dissolved oxygen content. The BOD value is then calculated from the results of the dissolved oxygen tests.

Consumables and replacement items

Required reagents

Description	Quantity/Test	Unit	Catalog number
BOD Nutrient Buffer Pillows, for 3 liters of dilution water	1 pillow	50/pkg	1486166

Required apparatus

Description	Quantity/Test	Unit	Catalog number
BOD Bottle, glass-stoppered, 300-mL	6	each	62100
BOD Bottle Cap	6	6/pkg	241906
Bottle, wash, 500-mL	1	each	62011
Clippers, large	1	each	96800
HQ40d meter	1	each	HQ40d
OR			
HQ30d meter	1	each	HQ30d
IntelliCAL LBOD probe	1	each	LBOD10101
Pipet, seriological:			
Pipet, serological, 1-mL	1	each	919002
Pipet, serological, 5-mL	1	each	53237
Pipet, serological, 10-mL	1	each	53238
Pipet Filler	1	each	1218900

Recommended standards

Description	Unit	Catalog number
BOD Standard Solution, Voluette® Ampule, 300-mg/L, 10-mL	16/pkg	1486510

Optional reagents and apparatus

Description	Unit	Catalog number
BOD Nutrient Buffer Pillows		
for 300 mL of dilution water	50/pkg	1486166
for 4 liters of dilution water	50/pkg	2436466
for 6 liters of dilution water	50/pkg	1486266
for 19 liters of dilution water	25/pkg	1486398
Buffer Solution, APHA, for BOD, pH 7.2, phosphate type	1 L	43153
Calcium Chloride Solution, APHA, for BOD	1 L	42853
Ferric Chloride Solution, APHA, for BOD	1 L	42953
Magnesium Sulfate Solution, APHA, for BOD	1 L	43053
Nitrification Inhibitor	35 g	253335
Dispenser Cap, for Nitrification Inhibitor	each	45901
Potassium Iodide Solution, 100-g/L	500 mL	1228949
Sodium Hydroxide, pellets, ACS	500 g	18734
Sodium Hydroxide Standard Solution, 1.000 N	100 mL MDB	104532
Sodium Thiosulfate Standard Solution, 0.025 N	1 L	35253
Starch Indicator Solution	100 mL MDB	34932
Sulfuric Acid Standard Solution, 0.020 N	1 L	20353
Sulfuric Acid Standard Solution, 1.000 N	1 L	127053
Up-Grade Kit, HQd Accessories, LBOD Probe	each	LBOD10130
Replacement LBOD Sensor cap, with I-button	each	5838000
Replacement Stirrer assembly	5/pkg	5850800
Field Kit, includes: protective glove, 2 standard probe holders and 5 120-mL sample cups	each	5825800
Keyboard (HQ40d meter only)	each	LZV582
Citizen PD-24 USB Handy printer, 115 VAC	each	5835800
Color Coded Probe Clips (5 color coded sets), 5 sets	10/pkg	5818400

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