6.1 Reagents

BioTector TOC TP analyzer uses following reagents:

I. **Acid:** 1.8 N Sulfuric Acid (H_2SO_4) Reagent containing 40mg/l Manganese Sulfate Monohydrate
II. **Base:** 1.2 N Sodium Hydroxide (NaOH) Reagent
III. **TN Cleaning:** 0.5 N HCl and 0.042 M Sodium Oxalate (NaOx) mixture solution
IV. **DI Water:** Deionized Water (0.1 – 0.5 µS/cm)
V. **TP Reagent:** Vanadate-Molybdate Reagent (contains 2.0 N HCl acid)
VI. **HCl Acid:** 3.0 N Hydrochloric Acid

BioTectors built with FMI Heavy Duty Circulation Pump require HCl Water, which is 0.04N Hydrochloric Acid solution. If there is no HCl WATER IN/OUT ports on BioTector, HCl Water solution is not required. Reagents should not contain high levels of organics, nitrates and phosphates. Ideally, the level of organics, nitrate and phosphate should be less than 100 µg/l (ppb) in the deionized water used to prepare TOC TP analyzer reagents. Phosphoric acid or Nitric acid cannot be used as an alternative acid in BioTector TOC TP analyzer.

Acid, Base, TN Cleaning solution, TP Reagent, HCl Acid and HCl Water reagents are stable up to 1 year. Table 7 below summarizes the total days each BioTector TOC TP Analyzer reagents lasts at various system configurations:

Table 7 BioTector TOC TP Analyzer Reagent Consumption

		TOTAL DAYS REAGENT LASTS		
REAGENTS	**Container Size (Liters)**	**Low Ranges* (< 500 mgC/l) (15 pulses) +**	**Medium Ranges* (500 – 2000 mgC/l) (23 pulses) +**	**High Ranges* (> 2000 mgC/l) (31 pulses) +**
Acid	25	35	23	17
Base	25	35	23	17
TN Cleaning	10	235	235	235
DI Water	10	70	70	70
TP Reagent	10	87	87	87
HCl Acid	10	87	87	87

*Low Ranges are typically less than 500 mgC/l. Medium Ranges are between 500 and 2000 mgC/l. High Ranges are typically greater than 2000 mgC/l.
+ The number of pulses acid and base reagents is injected every analysis cycle.

Above table is derived from several on-line operation parameters such as 100% on-line time. Note that TN Cleaning, DI Water, TP Reagent, HCl Acid and HCl Water consumptions are independent of BioTector range. A 10 liters HCl Water solution container typically lasts 38 days.

Recommended bunds (reagent spill trays) to contain above quantity reagents are 2x 50 Liters. If HCl Water solution is used, an additional 25 Liters reagent spill tray is also recommended.

6.1.1 Sulfuric Acid Reagent

There are a number of factors which affect the purity of the sulfuric acid solution used by the BioTector. These can take the form of inorganic or organic contamination. Inorganic contamination does not generally occur in concentrated acids, but there could be some dissolved CO_2 present in the deionised water, which is used to make up the acid solution. To remove any CO_2 dissolved in the deionised water used to make up the acid solution, sparge the water with a CO_2 free gas such as Nitrogen.

Organic contamination has two main sources: Organics, which are present in the concentrated sulfuric acid, or dissolved organics in the water used to make up the solution. The manufacturer will specify the level of organics in the concentrated sulfuric acid. For the BioTector, this should be as low as possible.

The procedure for making up sulfuric acid solutions from concentrated acid is as follows:

Mixing Procedure for the Preparation of 1.8N Sulfuric Acid Solution containing 40mg/l Manganese Sulfate Monohydrate:

Concentrated sulfuric acid is dangerous. The preparation of sulfuric acid solutions should only be carried out by persons properly trained in the handling of chemicals.

NEVER POUR WATER INTO ACID, ALWAYS POUR ACID INTO WATER!

Use eye protection and gloves.

A 1.8 N solution of sulfuric acid consists of 88.27 g (48 ml) of 100% sulfuric acid per liter of solution. Typically, concentrated sulfuric acid has purities of 96-98%. The procedures for both 1 liter and 25 liters of acid reagents are described below. The instructions below assume that the sulfuric acid used has a purity of 98%, which is the standard commercial purity of sulfuric acid.

1. To prepare an acid solution, always use deionised water, free from organic and inorganic carbon. The conductivity of the deionised water used should be less than 0.5 µS/cm.
2. Blow through the deionised water with a CO_2 free gas such as Nitrogen or Oxygen, to purge the water of any dissolved CO_2.
3. Fill 90% of the container with deionised water.
4. a) In order to prepare 1 liter of 1.8 Normal acid solution, add 90 grams (49 ml) of 98% purity sulfuric acid. Mix gently and add enough deionised water to make it exactly 1 liter.
   b) To prepare a 25 liters acid solution, add 2250 grams (1225 ml) of 98% purity sulfuric acid in stages. Adding the sulfuric acid in stages will prevent the solution from heating up. Gently mix and then add enough deionised water to make it exactly 25 liters.
5. Seal the container.
6. Gently shake the container to mix the acid with the water.
7. Add enough Manganese Sulphate Monohydrate catalyst ($MnSO_4.H_2O$), so that the acid reagent will contain 40 mg/l (mg per litre) of $MnSO_4.H_2O$. For instance, for a 25 litres acid solution, add 1 gram of $MnSO_4.H_2O$ and mix well.
8. Allow the container to stand for one hour, and gently shake again to ensure a good mix.

Control of Normality:

In order to check the Normality of the acid solution prepared, take a 12 ml sample from the prepared 1.8 N sulfuric acid solution. Add 2-3 drops phenolphthalein and just enough 1.2 N sodium hydroxide until the color of the mix becomes red. If the normality is correct, 18 ml of 1.2 N sodium hydroxide will neutralize 12 ml of 1.8 N sulfuric acid.

Calculations for Different Purities of Sulfuric Acid:

Table 8 below tabulates the required amounts of sulfuric acid with different purities.

Table 8 Amounts of concentrated sulfuric acid required to prepare 1 and 25 liters of 1.8 N acid solutions at various purities.

	1 Liter		25 Liters	
% Purity of Sulfuric Acid	**(ml)**	**(grams)**	**(ml)**	**(grams)**
100	48.00	88.27	1200	2207
99	48.48	89.16	1212	2229
98	48.98	90.07	1224	2252
97	49.48	91.00	1237	2275
96	50.00	91.95	1250	2299

6.1.2 Sodium Hydroxide Reagent

There are a number of factors which affect the purity of the sodium hydroxide solution used by the BioTector. These can take the form of inorganic or organic contamination. Inorganic contamination has two main sources: Sodium Carbonate (Na_2CO_3), which is present in the concentrated sodium hydroxide, or dissolved CO_2, which can be in the water used to make up the solution. The level of Na_2CO_3 in the concentrated sodium hydroxide will be specified by the manufacturer. For the BioTector, this should be 0.1% or lower. If possible the concentrated sodium hydroxide solution should be obtained from approved manufacturers (e.g. Sigma Aldrich and Fisher Scientific). Carbonates appears to be the main cause of contamination found in sodium hydroxide solutions, and generally the level of Na_2CO_3 is found to be lower in concentrated liquid sodium hydroxide than in sodium hydroxide pellets. Therefore, the use of sodium hydroxide pellets should be avoided when preparing the BioTector sodium hydroxide reagent.

To remove any CO_2 dissolved in the deionised water used to make up the sodium hydroxide solution, sparge the water with a CO_2 free gas, for example nitrogen. Ideally, the level of organics, nitrate and phosphate should be less than 100µg/l (ppb). If the conductivity of the deionised water used is less than 0.5µS/cm and the carbon content of deionised water is not more than 100µg/l (ppb), the CO_2 gas sparging procedure is not necessary.

Mixing Procedure for the preparation of 1.2N Sodium Hydroxide solution, from 50% concentrated Sodium Hydroxide solution:

Concentrated sodium hydroxide is dangerous. The preparation of sodium hydroxide solutions should only be carried out by persons properly trained in the handling of chemicals.

Use eye protection and gloves.

A 1.2N solution of sodium hydroxide consists of 48g of sodium hydroxide per liter of solution. Therefore, when using 50% concentrated sodium hydroxide, 96g of concentrate is required per liter. The procedures for both 1 liter and 25 liters of base reagents are described below.

It is important to purge the deionised water (used to make sodium hydroxide base reagent) with a CO_2 free gas prior to the mixing procedure.
As it is not possible to purge the carbonates (CO_3^-) from the base reagent by means of any CO_2 free gas, prepared base reagent should never be purged further with any gas after preparation.
When mixing the sodium hydroxide solution, care should be taken to minimize the amount of atmospheric CO_2 gas being absorbed in the solution. The base container should be fitted with a CO_2 filter to prevent the reagent coming in contact with air. Failure to do so will result in increased background CO_2 readings.

1. Use deionised water, free from organic and inorganic carbon. The conductivity of the deionised water used should be less than 0.5 µS/cm.
2. Fill 90% of the container with deionised water.
3. Blow through the deionised water with a CO_2 free gas such as Nitrogen or Oxygen, to purge the water of any dissolved CO_2.
4. a) In order to prepare 1 liter of 1.2 Normal base solution, add 96 grams of 50% purity sodium hydroxide. Mix gently and add enough deionised water to make it exactly 1 liter.
   b) To prepare a 25 liters base solution, add 2400 grams of 50% purity sodium hydroxide in stages. Adding the sodium hydroxide in stages will prevent the solution from heating up. Gently mix and then add enough deionised water to make it exactly 25 liters.
5. Seal the container.
6. Gently shake the container to mix the base with the water.
7. Allow the container to stand for one hour, and gently shake again to ensure a good mix.

If the base reagent is not mixed fully, then a highly concentrated layer will form in the bottom of the container. As the BioTector takes the base from the bottom of the container, this may create problems during operation, as the base will be too strong relative to the acid. Always mix the base solution for a second time, about 1 hour after it has been manufactured to ensure that it is completely mixed.

Control of Normality

Use a 10 ml sample from the sodium hydroxide container. Add 2-3 drops phenolphthalein and just enough 1.0N hydrochloric acid until it turns clear. If the normality is correct, 12ml of 1.0N hydrochloric acid will neutralize 10ml of 1.2N sodium hydroxide.

6.1.2.1 Sulfuric Acid Strength Testing Procedure

The procedure below describes a simple technique for checking the relative strength of the acid reagent.

Use eye protection and gloves.

In order to check the absolute strength of the acid reagent, a base standard of known strength (1.2N) will be required. The basis for the test is as follows: As the acid being tested is 1.8N, and the base is 1.2N, then 12ml of 1.8N acid mixed with 18ml of 1.2N base should give a pH of 7.

1. In a container, place 12ml of 1.8N acid. Check the pH. It should be 0pH.
2. Slowly add 16ml of 1.2N base. Check the pH. It should be ~1pH.
3. Add 1ml of 1.2N base, (total 17ml of base). Check the pH. It should be ~2pH.
4. Add 1ml of 1.2N base, (total 18ml of base). Check the pH. It should be ~7pH.
5. Add 1ml of 1.2N base, (total 19ml of base). Check the pH. It should be ~12pH.
6. Add 1ml of 1.2N base, (total 20ml of base). Check the pH. It should be ~13pH.

6.1.2.2 Sodium Hydroxide Strength Testing Procedure

The procedure below describes a simple technique for checking the relative strength of the base reagent.

Use eye protection and gloves.

In order to check the absolute strength of the base reagent, an acid standard of known strength (1.8N) will be required. The basis for the test is as follows: As the acid is 1.8N, and the base being tested is 1.2N, then 12ml of 1.8N acid mixed with 18ml of 1.2N base should give a pH of 7.

1. In a container, place 18ml of 1.2N base. Check the pH. It should be 14pH.
2. Slowly add 10ml of 1.8N acid. Check the pH. It should be ~13pH.
3. Add 1ml of 1.8N acid, (total 11ml of acid). Check the pH. It should be ~12pH.
4. Add 1ml of 1.8N acid, (total 12ml of acid). Check the pH. It should be ~7pH.
5. Add 1ml of 1.8N acid, (total 13ml of acid). Check the pH. It should be ~2pH.
6. Add 1ml of 1.8N acid, (total 14ml of acid). Check the pH. It should be ~1pH.

6.1.3 HCl Water Solution

In BioTectors built with FMI Heavy Duty Circulation Pump, a weak, 0.04N, Hydrochloric Acid solution (HCl Water) is used as a lubrication solution for the pump head.

Preparation of 0.04 Normal HCl Water Solution:

Concentrated hydrochloric acid is dangerous. Use eye protection and gloves. The preparation of hydrochloric acid solutions should only be carried out by persons properly trained in the handling of chemicals.

NEVER POUR WATER INTO ACID, ALWAYS POUR ACID INTO WATER!

Use eye protection and gloves.

A 0.04 N Hydrochloric acid solution consists 1.46 grams of Hydrochloric acid per liter of solution. Typically, concentrated Hydrochloric acid has purities of 32-37%. The instructions below are described to prepare 1 liter and 25 liters of 0.04 N Hydrochloric acid solution using both 32% and 37% concentrated Hydrochloric acid solutions.

1. To prepare the acid solution, always use deionised water, free from organic and inorganic carbon.
2. Fill 90% of the container with deionised water.
    a. To prepare 1 liter of 0.04 N Hydrochloric acid solution, add 3.6 grams (or 3 ml) of 37% concentrated Hydrochloric acid. Mix gently and add enough deionised water to make it exactly 1 liter.
    b. To prepare 1 liter of 0.04 N Hydrochloric acid solution, add 4.2 grams (or 3.5 ml) of 32% concentrated Hydrochloric acid. Mix gently and add enough deionised water to make it exactly 1 liter.
    c. To prepare 25 liters of 0.04 N Hydrochloric acid solution, add 90 grams (or 75 ml) of 37% concentrated Hydrochloric acid in stages. Adding the HCl in stages will prevent the solution from heating up. Mix gently and add enough deionised water to make it exactly 25 liters.
    d. To prepare 25 liters of 0.04 N Hydrochloric acid solution, add 105 grams (or 87.5 ml) of 32% concentrated Hydrochloric acid in stages. Adding the HCl in stages will prevent the solution from heating up. Mix gently and add enough deionised water to make it exactly 25 liters.
3. Seal the container.
4. Gently shake the container to mix the acid with the water.

6.1.4 TN Cleaning Solution

TN Cleaning solution is a mixture of 0.5 N Hydrochloric Acid and 0.042 M Sodium Oxalate. 0.042 M Sodium Oxalate contains 1000 mgC/l. Therefore, the chemicals required to prepare TN Cleaning Solution are as follows:

- 32% (or alternatively 37%) concentrated Hydrochloric Acid (HCl)
- 99.5% pure Sodium Oxalate (NaOx) salt
- Deionised water.

Preparation of TN Cleaning Solution:

Concentrated hydrochloric acid is dangerous. Use eye protection and gloves. The preparation of hydrochloric acid solutions should only be carried out by persons properly trained in the handling of chemicals.

NEVER POUR WATER INTO ACID, ALWAYS POUR ACID INTO WATER!

Use eye protection and gloves.

i) Fill 90% of a 1000 ml (±0.4 ml) long neck flask with deionised water.
ii) Weigh 5.61 grams of (99.5%) sodium oxalate and add it into the flask.
iii) Mix it thoroughly until all sodium oxalate salt dissolves in solution.
iv) Weigh 57.0 grams of (32% w/w) concentrated hydrochloric acid in an Erlenmeyer flask. Alternatively, weigh 49.3 grams of (37% w/w) concentrated hydrochloric acid in an Erlenmeyer flask.
v) Add the hydrochloric acid into the flask.
vi) Add enough deionised water to make it exactly 1 liter. Mix the solution thoroughly. The pH of the solution should be less than pH 1.
vii) The prepared TN Cleaning solution is stable up to 1 year.

Table 9 below summarizes the quantities of hydrochloric acid and sodium oxalate required to prepare various amounts of cleaning solutions.

Table 9 Quantities of HCl (32% or alternatively 37%) and NaOx (99.5%) required to prepare TN Cleaning Solution:

Amount of TN Cleaning Solution (Liters)	Quantity of HCl (32%w/w)* (Grams)	Alternative Quantity of HCl (37%w/w)* (Grams)	Quantity of NaOx (99.5%)* (Grams)
1	57.0	49.3	5.61
5	285.2	246.6	28.04
10	570.3	493.2	56.07
15	855.5	739.8	84.11
20	1140.6	986.4	112.14
25	1425.8	1233.0	140.18
30	1710.9	1479.6	168.21

*The displayed quantities change with the % purity of the chemicals.

6.1.5 TP Reagent

The chemicals required to prepare TP Reagent (Vanadate-Molybdate Reagent) are as follows:

- 32% (or alternatively 37%) concentrated Hydrochloric Acid (HCl)
- 99% pure Ammonium Heptamolybdate Tetrahydrate [$(NH_4)6Mo_7O_{24}.\ 4H_2O$]
- 99% pure Ammonium Metavanadate (NH_4VO_3)
- Deionised water. The level of organics, nitrate and phosphate in deionised water should be less than 100 μg/l (ppb).

The instructions below assume that the hydrochloric acid used has a purity of either 32% or 37%, which are the standard commercial purity of hydrochloric acid. The procedures described below covers the preparation of both 1 liter and 25 liters of TP reagent.

Preparation of TP Reagent:

Concentrated hydrochloric acid is dangerous. Use eye protection and gloves. The preparation of hydrochloric acid solutions should only be carried out by persons properly trained in the handling of chemicals.

NEVER POUR WATER INTO ACID, ALWAYS POUR ACID INTO WATER!

Use eye protection and gloves.

To prepare 1 liter of TP reagent using 32% HCl:

i) Prepare 6 M Hydrochloric Acid (HCl) solution by adding 683.4 grams (32% w/w) HCl in deionised water and dilute it to 1 liter.

ii) In order to prepare the TP reagent, first prepare SOLUTION A by dissolving 25 grams of 99% pure Ammonium Heptamolybdate Tetrahydrate [$(NH_4)6Mo_7O_{24}.\ 4H_2O$] in deionised water and dilute it to 300 ml.

iii) Prepare SOLUTION B by dissolving 2.5 grams of 99% pure Ammonium Metavanadate (NH_4VO_3) by heating to boil in 300 ml of distilled water. Cool solution B to room temperature.

iv) Prepare SOLUTION C by adding 330 ml of 6 M HCl into solution B. Then cool this solution to room temperature as well.

v) Pour solution A into solution C, mix well, and then dilute to exactly 1 liter by adding enough deionised water.

vi) The prepared TP reagent, which contains approximately 2 N HCl acid, is stable up to 1 year.

To prepare 25 liters of TP reagent using 32% HCl:

i) Prepare 6 M Hydrochloric Acid (HCl) solution by adding 6834 grams (32% w/w) HCl in deionised water and dilute it to 10 liters.

ii) In order to prepare the TP reagent, first prepare SOLUTION A by dissolving 625 grams of 99% pure Ammonium Heptamolybdate Tetrahydrate [$(NH_4)6Mo_7O_{24}$. $4H_2O$] in deionised water and dilute it to 7500 ml (7.5 liters).

iii) Prepare SOLUTION B by dissolving 62.5 grams of 99% pure Ammonium Metavanadate (NH_4VO_3) by heating to boil in 7500 ml of distilled water. Cool solution B to room temperature.

iv) Prepare SOLUTION C by adding 8250 ml of 6 M HCl into solution B. Then cool this solution to room temperature as well.

v) Pour solution A into solution C, mix well, and then dilute to exactly 25 liters by adding enough deionised water.

vi) The prepared TP reagent, which contains approximately 2 N HCl acid, is stable up to 1 year.

To prepare 1 liter of TP reagent using 37% HCl:

i) Prepare 6 M Hydrochloric Acid (HCl) solution by adding 591.1 grams (37% w/w) HCl in deionised water and dilute it to 1 liter.

ii) In order to prepare the TP reagent, first prepare SOLUTION A by dissolving 25 grams of 99% pure Ammonium Heptamolybdate Tetrahydrate [$(NH_4)6Mo_7O_{24}$. $4H_2O$] in deionised water and dilute it to 300 ml.

iii) Prepare SOLUTION B by dissolving 2.5 grams of 99% pure Ammonium Metavanadate (NH_4VO_3) by heating to boil in 300 ml of distilled water. Cool solution B to room temperature.

iv) Prepare SOLUTION C by adding 330 ml of 6 M HCl into solution B. Then cool this solution to room temperature as well.

v) Pour solution A into solution C, mix well, and then dilute to exactly 1 liter by adding enough deionised water.

vi) The prepared TP reagent, which contains approximately 2 N HCl acid, is stable up to 1 year.

To prepare 25 liters of TP reagent using 37% HCl:

i) Prepare 6 M Hydrochloric Acid (HCl) solution by adding 5911 grams (37% w/w) HCl in deionised water and dilute it to 10 litres.

ii) In order to prepare the TP reagent, first prepare SOLUTION A by dissolving 625 grams of 99% pure Ammonium Heptamolybdate Tetrahydrate [$(NH_4)6Mo_7O_{24}$. $4H_2O$] in deionised water and dilute it to 7500 ml (7.5 liters).

iii) Prepare SOLUTION B by dissolving 62.5 grams of 99% pure Ammonium Metavanadate (NH_4VO_3) by heating to boil in 7500 ml of distilled water. Cool solution B to room temperature.

iv) Prepare SOLUTION C by adding 8250 ml of 6 M HCl into solution B. Then cool this solution to room temperature as well.

v) Pour solution A into solution C, mix well, and then dilute to exactly 25 liters by adding enough deionised water.

vi) The prepared TP reagent, which contains approximately 2 N HCl acid, is stable up to 1 year.

6.1.6 HCl Acid Reagent

The procedures below describes the preparation of both 1 liter and 25 liters of HCl Acid reagent using 32% or 37% concentrated hydrochloric acid.

Preparation of 3 Normal HCl Acid Reagent:

Concentrated hydrochloric acid is dangerous. Use eye protection and gloves. The preparation of hydrochloric acid solutions should only be carried out by persons properly trained in the handling of chemicals.

NEVER POUR WATER INTO ACID, ALWAYS POUR ACID INTO WATER!

Use eye protection and gloves.

To prepare 3 N HCl Acid reagent using 32% HCl:

i) To prepare 1 liter of 3 Normal Hydrochloric Acid, add 341.7 grams of (32% w/w) HCl in deionised water and add enough deionised water to make the solution exactly 1 liter.
ii) To prepare 25 liters of 3 Normal Hydrochloric Acid, add 8543 grams of (32% w/w) HCl in deionised water in stages. Add enough deionised water to make the solution exactly 25 liters.
iii) Mix the solution thoroughly.

To prepare 3 N HCl Acid reagent using 37% HCl:

i) To prepare 1 liter of 3 Normal Hydrochloric Acid, add 295.5 grams of (37% w/w) HCl in deionised water and add enough deionised water to make the solution exactly 1 liter.
ii) To prepare 25 liters of 3 Normal Hydrochloric Acid, add 7389 grams of (37% w/w) HCl in deionised water in stages. Add enough deionised water to make the solution exactly 25 liters.
iii) Mix the solution thoroughly.

6.1.7 Deionised Water

- The deionised water used in BioTector must be reagent grade.
- The conductivity of the deionised water used should be less than 0.5 µS/cm.
- The organic carbon content of deionised water should not be more than 100 µg/l (ppb).

The level of nitrate and phosphate in DI Water should also be less than 100 µg/l (ppb).