

A stylized graphic of an Erlenmeyer flask, rendered in blue, green, and light green colors. The flask is positioned centrally on the page, with its neck and rim extending towards the top. The body of the flask is a thick, rounded shape. The colors transition from light green on the left to dark blue on the right.

**LABSTRONG
CORPORATION**

**ASTM TYPE II REAGENT GRADE WATER:
AN ANALYSIS OF DISTILLATION**

Table of Contents

INTRODUCTION:	3
ASTM TYPE II REAGENT GRADE WATER:	3
DISTILLATION PRODUCTION PROCESS:	5
BENEFITS OF DISTILLATION:	6
Broad Removal Capabilities:	6
Consumables Not Required:	6
Easy to Start Up or Shut Down:	6
Sterile:	6
Simple Operation:	7
Simple Set-Up:	7
ASTM TYPE II DISTILLED REAGENT GRADE WATER APPLICATIONS:	7
Biotechnology Methods:	7
Water Analysis and General Chemistry Methods:	7
Clinical Lab Methods:	7
General Laboratory Equipment:	8
Hydroponics and Aquaculture:	8
Fixatives:	8
Solid Phase Extraction and Liquid Chromatography Techniques:	8
Feed Water for Type 1 Ultrapure Water Purification System:	8
Lead Acid Batteries:	9
In Place of Pre-Bottled Water:	9
Methods Not Requiring Type I RGW:	9
CONCLUSION:	9
REFERENCE LIST:	10

Table of Figures

Figure 1: Processes for Reagent Water Production.....	4
Figure 2: Example of Laboratory Distillation System	5
Figure 3: Distillation Production Process	5
Figure 4: Water Purification System Removal Capabilities	6
Figure 5: Deionization & Reverse Osmosis System Consumables	6
Figure 6: Dishwasher	8
Figure 7: Autoclave.....	8
Figure 8: Type I RGW System	8
Figure 9: Pre-Bottled Water.....	9

INTRODUCTION:

The most vital piece of equipment in any laboratory is the water purification system utilized to create reagent grade water. Water purification systems provide pure water critical for experiments and other analytical applications. Many individuals take water purification systems for granted, but they should not be because the presence of impurities in water can have adverse effects on any procedure. In reality, the laboratory reagent grade water system is the most important “instrument” in the lab. Reagent grade water quality will affect the precision and accuracy of every other instrument or test performed in the lab.

Various water purification technologies can remove impurities threatening research. One technology used to purify water is distillation. Distillation has been one of the most commonly used forms of purifying water for centuries. Science, engineering, and industrial settings use distilled water for a variety of applications. This paper will examine ASTM Type II reagent grade water standards, how water is purified using distillation, benefits of distilled water, and applications requiring distilled water.

ASTM TYPE II REAGENT GRADE WATER:

The American Society for Testing and Materials, known as ASTM International, is a voluntary standards organization who publishes standards and specifications of quality for a multitude of materials. The ASTM Standards for laboratory reagent grade water (RGW) contain the highest quality standard requirements of any agency published. Reagent grade water “is used specifically as a component of an analytical measurement process and meets or exceeds the specifications for these waters” (ASTM D1193-06, 2011).

The ASTM Standard Specification for Reagent Water specifies four types of reagent grade waters, which include ASTM Type I, II, III, and IV (ASTM D1193-06, 2011) (Figure 1). The four types of reagent grade water “may be produced with alternate technologies as long as the appropriate constituent specifications are met and that water so produced has been shown to be appropriate for the application where the use of such water is specified” (ASTM D1193-06, 2011). One technology used to produce Type II reagent grade water is distillation.

Until recently, ASTM required Type II reagent grade water be produced only by distillation. As of the latest D1193 designation, ASTM currently allows other means of purification for Type II reagent grade water as long as all specifications are met (ASTM D1193-06, 2011) (Figure 1). When selecting a technology other than distillation, one must take into account the potential impact of contaminants not removed from the purified water such as pyrogens and microorganisms. In addition, one must take into account the potential impact on water purity as consumables exhaust. For these reasons, laboratories typically prefer the original standard, reliable, and consistent production process of distillation to produce Type II reagent grade water.

Type	Grade	Production Process ^{A,B,C,D}	$\mu\text{S}/\text{cm}^E$ (max)	$\text{M}\Omega\cdot\text{cm}^F$ (min)	pH^G	TOC $\mu\text{g}/\text{L}^H$ (max)	Sodium $\mu\text{g}/\text{L}^I$ (max)	Chloride $\mu\text{g}/\text{L}^J$ (max)	Total Silica $\mu\text{g}/\text{L}$ (max)	HBC ^K cfu/mL (max)	Endotoxin, EU/mL ^L (max)
I		Purify to 20 $\mu\text{S}/\text{cm}$ by dist. or equiv., followed by mixed bed DI, 0.2 μm filtration ^A	0.0555	18		50	1	1	3		
I	A	Purify to 20 $\mu\text{S}/\text{cm}$ by dist. or equiv., followed by mixed bed DI, 0.2 μm filtration ^A	0.0555	18		50	1	1	3	10/1000	0.03
I	B	Purify to 20 $\mu\text{S}/\text{cm}$ by dist. or equiv., followed by mixed bed DI, 0.2 μm filtration ^A	0.0555	18		50	1	1	3	10/100	0.25
I	C	Purify to 20 $\mu\text{S}/\text{cm}$ by dist. or equiv., followed by mixed bed DI, 0.2 μm filtration ^A	0.0555	18		50	1	1	3	100/10	
II		Distillation ^B	1.0	1.0		50	5	5	3		
II	A	Distillation ^B	1.0	1.0		50	5	5	3	10/1000	0.03
II	B	Distillation ^B	1.0	1.0		50	5	5	3	10/100	0.25
II	C	Distillation ^B	1.0	1.0		50	5	5	3	100/10	
III		Distillation, DI, EDI, and/or RO, followed by 0.45 μm filtration. ^C	0.25	4.0		200	10	10	500		
III	A	Distillation, DI, EDI, and/or RO, followed by 0.45 μm filtration. ^C	0.25	4.0		200	10	10	500	10/1000	0.03
III	B	Distillation, DI, EDI, and/or RO, followed by 0.45 μm filtration. ^C	0.25	4.0		200	10	10	500	10/100	0.25
III	C	Distillation, DI, EDI, and/or RO, followed by 0.45 μm filtration. ^C	0.25	4.0		200	10	10	500	1000/100	
IV		Distillation, DI, EDI, and/or RO. ^D	5.0	0.2	5.0 to 8.0		50	50			
IV	A	Distillation, DI, EDI, and/or RO. ^D	5.0	0.2	5.0 to 8.0		50	50		10/1000	0.03
IV	B	Distillation, DI, EDI, and/or RO. ^D	5.0	0.2	5.0 to 8.0		50	50		10/100	0.25
IV	C	Distillation, DI, EDI, and/or RO. ^D	5.0	0.2	5.0 to 8.0		50	50		100/10	

Figure 1: Processes for Reagent Water Production

DISTILLATION PRODUCTION PROCESS:

The production process of distillation is truly unique from other production processes. During distillation, water is removed from the impurities rather than the impurities from the water. Distillation physically separates the water from the contaminated or high salt source water, leaving dissolved salts and other debris behind.

For laboratory grade water, distillation works by heating or boiling liquid water to change the liquid into the vapor phase and then cooling the vapor back to liquid water (condensation) for collection (Figure 3). The process of distillation begins by heating water in a heating vessel or boiler of a still. When heated in the boiler, the water undergoes a phase change from a liquid to a vapor. The vapor rises into a condenser chamber, leaving impurities in the heating vessel or boiler. The vapor cools in the condenser and then changes from a vapor back to a liquid, known as distillate.

The distillate water must have a conductivity of less than $1.0 \mu\text{S}/\text{cm}$ ($>1.0 \text{M}\Omega \cdot \text{cm}$) at 25°C to meet Type II reagent grade water requirements. In addition, the distillate water must have a maximum TOC $\mu\text{g}/\text{L}$ of $50 \mu\text{g}/\text{L}$, maximum sodium of $5 \mu\text{g}/\text{L}$, maximum chloride of $5 \mu\text{g}/\text{L}$, and maximum total silica of $3 \mu\text{g}/\text{L}$. Heterotrophic Bacteria Count (HBC) cfu/mL and bacterial endotoxin EU/mL level requirements vary upon Type II Grade requirements (Figure 1) (ASTM D1193-06, 2011).



Figure 2: Example of Laboratory Distillation System

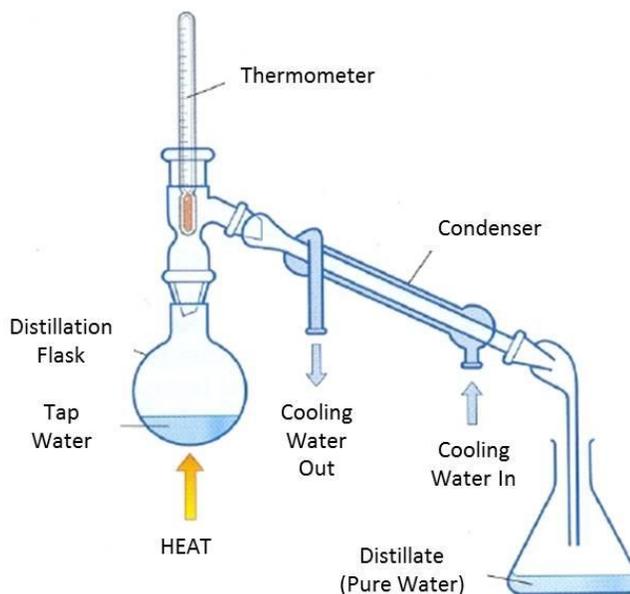


Figure 3: Distillation Production Process

BENEFITS OF DISTILLATION:

Broad Removal Capabilities:

Distillation is ideal for Type II applications because it offers the broadest removal capabilities of any single form of water purification (Figure 4). Distillation can remove inorganic ions, dissolved gases, organics, particles, bacteria, and pyrogens from the water leaving high quality Type II water suitable for a variety of lab applications.

		Water Purification Technology					
		Distillation	Reverse Osmosis	Ultrafiltration	Adsorption	Filtration	Deionization
Water Impurities	Pyrogens	●●	●●	●●	●		
	Bacteria	●●	●●	●●		●●	
	Particles	●●	●●	●●		●●	
	Inorganic Ions	●●	●				●●
	Organics	●	●		●●		
	Dissolved Gases	●			●		●●
	Nucleases			●	●		
Total Purity Number		10	8	7	5	4	4

●● EXCELLENT WATER PURIFICATION
 ● GOOD WATER PURIFICATION
 ● POOR WATER PURIFICATION

Figure 4: Water Purification System Removal Capabilities

Consistent Quality of Pure Product Water:

Unlike other water purification technologies, distillation consistently produces water free of impurities. Deionized product water quality quickly drops off as consumables become exhausted (Figure 5). Reverse osmosis product water quality fluctuates and output decreases as consumable membranes and prefilters become exhausted (Figure 5).

Consumables Not Required:

Distillation typically does not require the use of consumables to operate. This eliminates concerns associated with costly filters or cartridges such as consumable shelf life, longevity, or stocking. Deionization requires continuous replacement of consumables such as cartridges, UV lamps, ultra-filters, pumps, and final filters (Figure 5). Reverse osmosis product water requires continuous replacement of membranes, pumps, and filters (Figure 5).



Figure 5: Deionization & Reverse Osmosis System Consumables

Easy to Start Up or Shut Down:

Distillation systems can easily be turned on or off for extended periods without the hassle of replacing consumables. Deionization and reverse osmosis water systems require the replacement of consumables if turned off for extended periods.

Sterile:

Stills sterilize themselves during operation. When on, the water temperature in the still rises to its boiling point and is maintained indefinitely. If the condenser chamber is properly designed, sterile pyrogen-free RGW can be produced. This is why distillation has been so important to biotech and pharmaceutical industries.

Simple Operation:

Distillation systems do not require pretesting of inlet water source or constant monitoring. Deionization and reverse osmosis water systems require constant monitoring and pretesting of inlet water to ensure reagent grade water is meeting Type II requirements.

Simple Set-Up:

Distillation is simple to set-up and does not require pretesting or prequalification of water sources. Deionization and reverse osmosis water systems require chemical testing of water. Deionization requires at least some type of pretreatment.

ASTM TYPE II DISTILLED REAGENT GRADE WATER APPLICATIONS:**Biotechnology Methods:**

Biotechnology methods require sterile pyrogen free water to prevent bacterial contamination and potential interference from pyrogens (bacterial endotoxins). Pyrogens can disrupt or destroy yields by changing gene expression. Pyrogens also interfere with drug discovery methods.

Water Analysis and General Chemistry Methods:

Water analysis and general chemistry methods require reagent grade water free of dissolved salts, bacteria, and organic compounds for most all test methods. Viable bacteria or spores in the reagent water can produce false high results (high oxygen demand) during biochemical oxygen demand (BOD) incubation. Bacteria, dissolved solids, and organic compounds may increase chemical oxygen demand (COD) in waste or environmental samples. High organic levels in RGW may interfere with Total Organic Carbon (TOC) tests by disrupting calibration curves and adding undetected TOC between rinse cycles.¹

General lab reagent creation requires water free of salts to insure quality control for all lab reagents. Water free of contaminants is required for pH buffer stability, titration solutions and most wet chemistries to ensure accuracy and precision in the lab. This includes calibration standard development and/ or concentrated sample dilution requirement. Type II water is suitable for major ion analyses of natural and potable water.

**Clinical Lab Methods:**

A number of application specific clinical lab methods require Type II RGW. The minimum quality for RGW is indicated in the actual published test methods. The Clinical and Laboratory Standards Institute (formerly NCCLS) now govern these methods. Type II ASTM RGW quality exceeds the previously published NCCLS Type II lab water specification.

¹ Pretreatment with activated carbon to remove lower boiling point organic compounds may be required depending on feed water quality. Activated carbon is also recommended to remove chlorine species to avoid carryover into the product water.

General Laboratory Equipment:

Most laboratory environmental chambers, autoclaves, dishwashers, and humidifiers recommend the use of Type II RGW. Type II water does not contain minerals that will form scale in heating equipment or on glassware and should not leave residue after evaporation.² Type II water is less aggressive towards wetted plumbing parts, pumps, and metal parts as compared to Type I water.

**Figure 6: Dishwasher***Credit: Labconco***Figure 7: Autoclave***Credit: Astell***Hydroponics and Aquaculture:**

Type II water provides a suitable base water to start with for hydroponics and aquaculture studies and small-scale production operations. For hydroponics, minerals, and nutrients can be added to the water in precise quantities with the knowledge that there are no added substances that could create a nutrient imbalance with plant growth. Fresh and saltwater can be created from scratch by accurately adding hardness minerals and other required salts to create an ideal environment for aquatic flora and fauna.

Fixatives:

Rinsing samples after fixation and for diluting fixation chemistries require the use of distilled water. Type II distilled RGW should exceed the requirements for most fixative techniques.

Solid Phase Extraction and Liquid Chromatography Techniques:

Depending on the specific application and detection ranges, Type II distilled water can be used for most forms of solid phase extraction and liquid chromatography techniques. Distilled water is used in Normal Phase extraction methods for sample prep, solid media rinsing and buffer solution mobile phase creation. In addition, distilled water may be used as the mobile phase in Reverse Phase extraction or chromatography due the polarity of the purified water relative to the solid media. Distilled water is also utilized for sample preparation and mobile phase solution creation for solid phase ion exchange methods.

Feed Water for Type 1 Ultrapure Water Purification System:

Type II distilled water is a preferred feed water source for Type I RGW systems or other purification technology based on ion exchange. Ion exchange resins have a limited capacity to purify water based on the salt content of the feed water source. Distilled water typically contains only carbon dioxide in the ionic form of carbonic acid.

Distilled water is also typically free of colloidal or other particles that can foul ion exchange resins. Using distilled water as a feed source to ion exchange systems can extend the usable operating capacity of the ion exchange resins by one to several orders of magnitude as compared to reverse osmosis or tap water feed sources. This can dramatically reduce the cost of Type 1 system consumables. Fresh distilled water is typically devoid of any viable bacteria, biologic fouling of the ion exchange resins and larger molecular weight organic compounds.

**Figure 8: Type I
RGW System***Credit: Thermo Scientific*

² Proper still and water storage maintenance is required. Lab storage containers may leach material into the water that could leave residue behind.

Lead Acid Batteries:

Lead acid batteries specify distilled water for topping off since the water contains no dissolved salts that could tend to neutralize the acid contained in the battery. If acid strength is reduced, battery life and power will also be reduced.

In Place of Pre-Bottled Water:

Pre-bottled water can be risky if used as a reagent grade water source. The age of the water is unknown. It is also likely unknown if the water met any of the Type II standards as produced let alone as purchased. Plastic bottles used for store purchased distilled water may contain plasticizers, antioxidants, UV blockers, mold lubricants and/or metal contaminants. Plasticizers used may be listed as potential endocrine disruptor substances that could interfere with biotech applications.

In addition, each time a bottle of water is opened it is exposed to the lab atmosphere that can allow the water to absorb any gases or fumes which may be present. Airborne spores from bacteria or fungi can also contaminate the water.

Distillation systems produce fresh water for use in labs. Storage vessels used for collecting distilled water are made of specialized plastic or glass designed to reduce or eliminate material leaching into the stored water. Properly designed storage vessels also contain breather filters to prevent airborne biota, particles, and organic fumes from entering the vessels. Vessel filters are also available to reduce CO₂ uptake in the stored water.



Figure 9: Pre-Bottled Water

Credit: W.W. Grainger, Inc.

Methods Not Requiring Type I RGW:

Type II distilled water can be utilized for most any general laboratory method not requiring Type I RGW. Other methods of producing Type II water can contain large amounts of bacteria, particles and organic carbon compounds that can breakthrough undetected causing contamination problems in the lab.

CONCLUSION:

Distillation is still the only accepted and preferred method of achieving Type II water, without requiring additional validation. Distillation assures consistent product water – not degrading as components exhaust. Distillation is sterile water that does not pass through bacterial contamination or pyrogen parts when properly designed. For these reasons, distillation has broad application acceptance and reliability.

REFERENCE LIST:

- Astell. (2015). *The Astell Front Loading Autoclave Range*. Retrieved April 16, 2015, from Astell:
<http://www.astell.com/swiftlock-front-lab-sterilizers/>
- ASTM D1193-06 (2011), Standard Specification for Reagent Water, ASTM International, West
Conshohocken, PA, 2011, www.astm.org
- Labconco. (2015). *FlaskScrubber Vantage Series Glassware Washers*. Retrieved April 16, 2015, from
Labconco: <http://www.labconco.com/product/flaskscrubber-vantage-series-glassware-washers/80>
- Thermo Fisher Scientific Inc. (2015). *Barnstead NANOpure*. Retrieved April 15, 2015, from Thermo
Scientific: <http://www.thermoscientific.com/en/product/barnstead-nanopure.html>
- W.W. Grainger, Inc. (2015). *Water, deionized (ASTM Type II), 20 L*. Retrieved April 16, 2015, from
W.W Grainger, Inc.: <http://www.grainger.com/product/LABCHEM-Water-4YMH3>