



# Wako

# Product Update

## BIOCHEMISTRY

### *Forensics Research Kits*

- *DNA extraction from bloodstain, hair, and nail*
- *Simple and quick semen detection*

*Research for protein degradation*

*Long-awaited prepared inulin solution, etc.*

## ANALYTICAL CHEMISTRY

*Complete line of pretreated columns  
for dioxins analysis!*

*Simple and quick analysis of  
residual chlorine*



Wako

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### DNA Extractor FM Kit

Cat. #295-58501 50 tests  
2-10°C

Identification of an individual by DNA analysis is a powerful method in forensic medicine. However, samples for forensic DNA analyses obtained at the scene are often insoluble materials such as body hairs, nails, saliva stains and blood stains, which usually contain quite limited amounts of DNA. Therefore, it is sometimes difficult to perform a DNA examination due to complicated hard isolation procedures or its insufficient recovery. This kit provides a rapid procedure for isolation of DNA from such rigid materials, overcoming the difficulties described above, by combining rapid solubilization of DNA from the rigid materials with our original NaI DNA extraction procedure without the use of harmful organic solvents such as phenol or chloroform.

#### [Assay Materials]

Body hair (2 pieces, 1cm or shorter), nail (0.5 mg or less), saliva stain and blood stain.

#### [Features]

1. Solubilizes a variety of tough hairs as body hair completely within several ten minutes.
2. Eliminates the need for harmful organic solvents such as phenol or chloroform.
3. Avoids loss of DNA from limited amounts of sample DNA, unlike solid phase.
4. DNA extraction by non-specific absorption of DNA to solid support.
5. Attains DNA purification with nearly 100% recovery by using NaI-DNA Extraction.
6. Ready to use DNA preparation obtained by the kit in DNA amplification as the polymerase chain reaction.
7. Provides a set of essential reagents for the procedure with the kit.



#### [Kit Contents (for 50 assays)]

1. Lysis Solution ----- 9.5 mL
2. Enzyme-activated Reagent (EAR) ---- 80 mg
3. Reconstitutin Solution for EAR ----- 0.5 mL
4. Protease ----- 10 mg
5. Sodium Iodine Solution----- 12.5 mL
6. Washing Solution (A) ----- 50 mL
7. Washing Solution (B) ----- 50 mL

PCR amplification of high DNA variation region of mitochondria using DNA extracted from hair shaft portion



Comparison of amplification efficiency of high DNA variation region of mitochondria by difference of staining properties

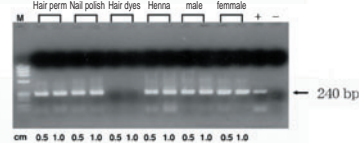
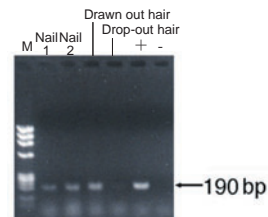


Fig.1: PCR amplification of hyper variable region of mitochondria DNA extracted from hair

PCR amplification of TH01



PCR amplification of high DNA variation region of mitochondria (from base No. 15997 to base No. 16401)

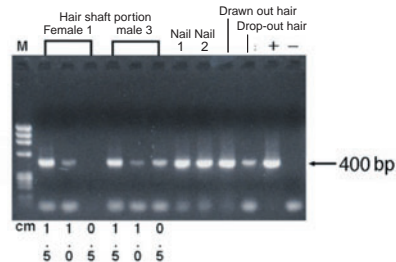


Fig.3: PCR amplification of short tandem repeat TH01of genom DNA and hyper variable of mitochondria DNA isolated from the nail and the root associated with hair

#### DNA Quantitation

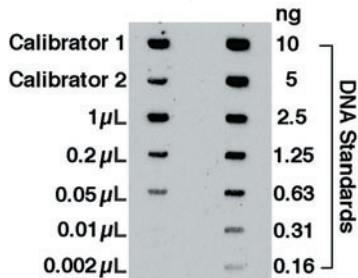


Fig.2 : Quantation results of DNA extracted from 1mL of blood diluted 1 to 500 times

These data are all provided by courtesy of Prof. Y. Seo, Department of Legal Medicine at Miyazaki Medical College.

#### [Reference]

1. Wang, L. *et al.*: *Nucleic Acids Res.*, **22**, 1774 (1994)

### SM Reagent

for Forensics research

Cat. # 297-58201 for 500 mL

2-10°C, in the dark

#### [Features]

- [1] The kit is extensively used for identifying acidic phosphatase originating from granula prostatica, which exists in a large quantity in human semen, and evidencing semen.
- [2] High sensitivity.
- [3] The quickly developed bright purple color is hard to degrade, is stable, and is kept well.
- [4] Both preparation method and usage are simple and easy.

#### [Kit Contents]

1. SM Reagent No. 1 ( $\alpha$ -Naphthylphosphoric acid) 1 bottle x 1 g
2. SM Reagent No. 2 (Diazonium o-dianisidine) 1 bottle x 2 g

#### [Detection method]

##### [1] Direct color reaction method

##### [2] Atomization method

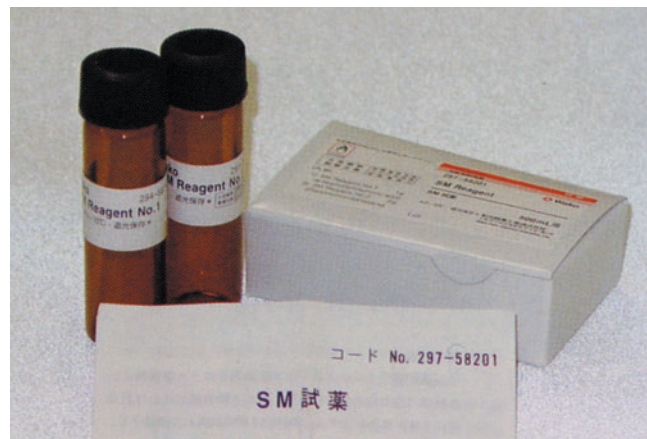
##### [3] Test paper method

#### [Note]

- The similar color reaction may be exhibited by acidic phosphatase contained in semen of order of primates such as monkeys, etc., human body fluid, plant and anaerobic bacteria, putrefactive bacteria, etc.
- It is not allowed to use the specimen to which the present test solution adheres for other purposes such as blood grouping, etc. as specimens.

#### [Reference]

- 1) Hirofumi Suyama, *et al.*: Prostatic Acid Phosphomoneesterase of the Japanese Monkey (*Macaca f. fuscata*), *Primates*, **9**, 141 (1968).
- 2) Kazue Ueno, *et al.*: Acid Phosphatase in *Clostridium perfringens*. A New Rapid and Simple Identification Method, *Jap. J. Microbiology*, **14** (2), 171 (1970).
- 3) Hideo Sawada, *et al.*: Studies on Human Prostatic Acid Phosphatase V. Isolation and Characterization of a Prostatic Acid Phosphatase Isozyme., *Chem. Pharm. Bull.*, **29**, (12), 3624 (1981).



#### [How to use]

##### Preparation of SM test solution

- 1) Dissolve 0.2 g of SM reagent No. 1 and 0.4 g of SM reagent No. 2 in 100 mL of 0.2 mol/L citrate buffer (pH 5.0) with stirring at room temperature.
- 2) After letting it stand in a cold and dark room for 30 minutes, filter precipitate, place the obtained amber solution in a light resistant bottle and store in a refrigerator (SM test solution).  
 (Note) This SM test solution can be used for one week by filtering precipitate even when any precipitate is generated. However, since the SM test solution is unstable to heat and light, prepare the required amount before use.aper).

#### [Of various detection methods]

- [1] **Direct color reaction method** (prepared reagent is directly added dropwise to the specimen)

Place specimen (use one piece of fiber for spot marks of fabric, etc.) on paraffin paper or white porcelain dish and add one drop of the SM test solution; then, bright purple color is developed immediately in the case of positive reaction.

(Note) This SM test solution can be used for one week by filtering precipitate even when any precipitate is generated. However, since the SM test solution is unstable to heat and light, prepare the required amount before use.aper).In the event that the specimen is liquid such as body fluid, etc., take a small amount into a small test tube, dilute with a small amount of the above citrate buffer, add the SM test solution dropwise, and observe the color reaction.In either case, judge the results by comparing with the blank test results.

- [2] **Atomization method** (prepared reagent is atomized and sprayed)

Place the SM test solution diluted 5 times with the above-mentioned citrate buffer in a glass spray bottle, and spray over a specimen (in the case of white fabric). If the spot mark is a semen spot, the whole attached portion turns purple. Use the SM test solution by preparing before use.

- [3] **Test paper method** (simple and quick semen detection method that has enabled the use of test paper as required by preparing the test paper)

##### 1) Preparation of test paper

Soak filter paper in SM test solution, immediately allow it to absorb excess test solution, and air-dry in a dark room; then, cut into appropriate sizes. When this is placed in a light resistant bottle and refrigerated, the filter paper can be used for 3 weeks.

##### 2) Semen identification method

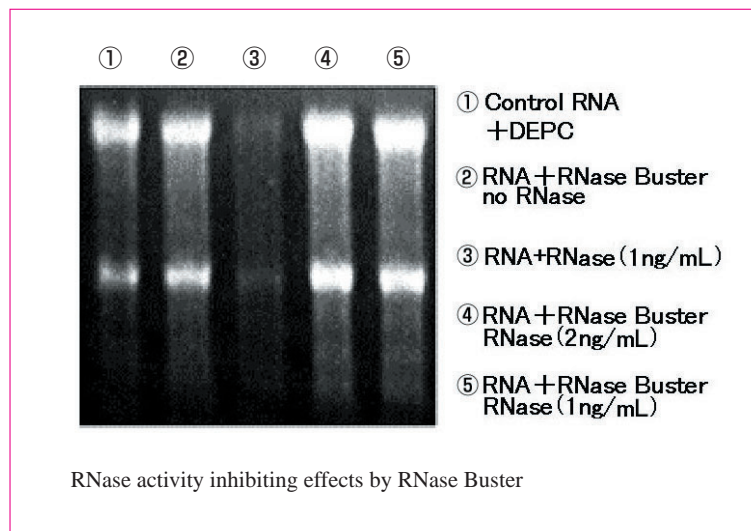
If the specimen is fabric, collect one piece of fiber, place it on a test paper, add one drop of the above-mentioned citrate buffer, and sandwich the specimen. If it is positive, the test paper at the specimen adhering portion immediately assumes purpose. In the case of body fluid or specimen adhering to the body surface, use test paper moistened with the citrate buffer in advance.

Protecting RNA from RNase contamination.

### RNase Buster

for Gene Investigation  
547-02281 10 mL

RNase Buster can easily inactivate RNase for solutions which cannot undergo DEPC treatment such as Tris Buffer, etc. A solution with RNase Buster added can have RNase repeatedly inactivated by heating even if RNase contamination occurs during operation.



After all mouse brain RNAs were dissolved with RNase Buster or dissolved with DEPC treated water, RNase Mix (RNase A, RNase T1) was added to achieve 2 ng/mL concentration.

The sample was incubated at 37°C for 17 hours after heating at 60°C for 20 minutes. RNA was detected by 1% agarose gel by EtBr dyeing.

#### [How to use]

1. Dilute RNase Buffer in buffer solution or solution so that the end concentration becomes 1%, and agitate.
2. Incubate at 60°C for 2 hours. By cooling to room temperature, the buffer solution and solution attain RNase-free condition.
3. If any re-contamination of RNase is suspected during operation, reheat at 60°C for 10-20 minutes.

## 3. physiologically active substance

No troublesome dissolution required! Low endotoxin!

### 20w/v% Inulin Solution (Low Molecular Fraction)

for Gene Investigation Biochemistry  
091-04853 2 mL×5  
095-04851 10 mL×5

RNase Buster can easily inactivate RNase for solutions which cannot undergo DEPC treatment such as Tris Buffer, etc. A solution with RNase Buster added can have RNase repeatedly inactivated by heating even if RNase contamination occurs during operation.

Inulin is a storage polysaccharide distributed primarily in aster-family plants. Inulin is voided in the kidney when administered in blood, but in such event, inulin freely penetrates glomerulus and is neither secreted nor reabsorbed in urinary tubule. Consequently, inulin is used as a reference material for measuring the glomerular filtration rate (GFR).

When inulin is administered, it must be made into an aqueous solution. The powder of this inulin provides properties which are difficult to dissolve in water and are likely to precipitate crystal even after dissolution. In addition, since commercially available inulin powder has a possibility of being contaminated by endotoxin which has exothermic action, an operation to remove this is needed, requiring extremely troublesome procedures for preparing the aqueous inulin solution.

Because 20w/v% Inulin Solution is a solution with endotoxin removed, in which inulin is adjusted to 20w/v% concentration, all you need to do is to dilute the product and use it as is.

#### [Features]

- Troublesome dissolution operation of inulin powder is no longer necessary.
- Low endotoxin (0.25 EU/mL or less)
- Autoclave-sterilized.

### a. Research for Protein Degradation

#### Ubiquitin-Proteasome System

It is known that the selective protein degradation mechanism functions to control the amount of specific proteins temporally and spatially and undertakes important roles in controlling higher order functions such as cell cycle, apoptosis, metabolism control, signal transduction, transcription control, immune response, etc. as well as in maintaining homeostasis such as stress response, protein quality control, etc., and furthermore, in various life activities such as fertilization, development, etc. In particular, the ubiquitin-proteasome system is an ATP-dependent protein degradation pathway which is important for various biological functions within the living body and is one of the most important selective protein degradation systems. In describing its stringent selectivity and quick degradation, it is not excessive to compare the ubiquitin-proteasome system to a "protein killer." Wako has a wide choice of the following ubiquitin-proteasome system-related reagents.

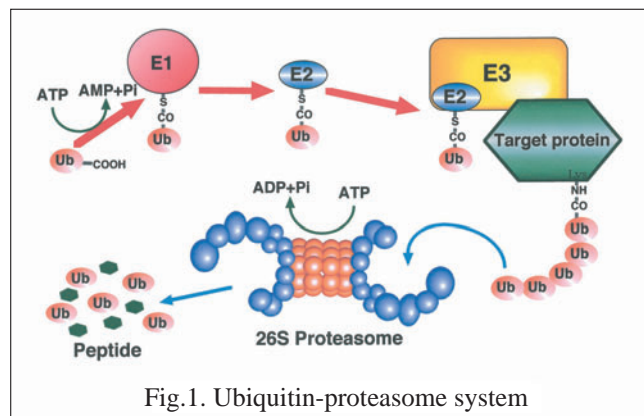


Fig.1. Ubiquitin-proteasome system

#### [Reference]

- 1) Morimoto and Tanaka, H.: *Wako-Junyaku Jiho*, **71**(2), 5-7 (2003).
- 2) Hershko, A. and Ciechanover, A.: *Ann. Rev. Biochem.*, **67**, 425-479 (1998).

Wako Cat. #	Description	Grade	Package Size	Condition
<b>E1 (Ubiquitin Activating Enzymes)</b>				
219-01111	Ubiquitin Activating Enzyme, Mouse, recombinant, Solution	for Cell Biology	25 $\mu$ g	-80°C
<b>E2 (Ubc: Ubiquitin Conjugating Enzymes)</b>				
213-01131	Ubiquitin Conjugating Enzyme, Ubc3, Human, recombinant, Solution	for Cell Biology	100 $\mu$ g	-80°C
215-01191	Ubiquitin Conjugating Enzyme, UbcH5a, Human, recombinant, Solution	for Cell Biology	100 $\mu$ g	-80°C
218-01201	Ubiquitin Conjugating Enzyme, UbcH5b, Human, recombinant, Solution	for Cell Biology	100 $\mu$ g	-80°C
216-01121	Ubiquitin Conjugating Enzyme, UbcH5c, Human, recombinant, Solution	for Cell Biology	100 $\mu$ g	-80°C
210-01141	Ubiquitin Conjugating Enzyme, (SUMO-1), Human, recombinant, Solution	for Cell Biology	100 $\mu$ g	-80°C
217-01151	Ubiquitin Conjugating Enzyme, Ubc12 (NEDD8), Human, recombinant, Solution	for Cell Biology	100 $\mu$ g	-80°C
<b>UBL (Ubiquitin-like Proteins)</b>				
199-12771	SUMO-1, Human, recombinant, Solution	for Cell Biology	200 $\mu$ g	-80°C
145-07621	NEDD8, Human, recombinant, Solution	for Cell Biology	100 $\mu$ g	-80°C
<b>Inhibitors</b>	In the ubiquitin-proteasome system that plays an important role in controlling cell functions, first of all, ubiquitin combines with the target protein and issues a protein degradation signal. Proteasome, which detects it, decomposes the target protein. The followings are inhibitors of those proteasomes.			
131-14011	MG-115 (Z-Leu-Leu-Nva-CHO)	for Biochemistry	5 mg	-20°C
138-14021	MG-132 (Z-Leu-Leu-Leu-CHO)	for Biochemistry	5 mg	-20°C
135-14031	MG-262 [Z-Leu-Leu-Leu-B(OH)2]	for Biochemistry	100 $\mu$ g	-20°C
215-01071	Ubiquitin Aldehyde	for Biochemistry	50 $\mu$ g	-20°C
333-43681	Lactacystin	Peptide Institute Inc.	0.2 mg	-20°C
031-18201	clasto-Lactacystin $\beta$ -Lactone	for Biochemistry	100 $\mu$ g	-20°C
058-06841	Epoxomicin	for Biochemistry	100 $\mu$ g	-20°C
<b>Substrates</b>	Substrates of 20S proteasome which have chymotrypsin-like protease activities. Using it in combination with inhibitor enables it to be used for research of specificity of proteasome activity.			
164-20511	20S Proteasome Fluorogenic Substrate	for Biochemistry	5 mg	-20°C
<b>Proteasomes</b>				
161-20521	20S Proteasome, from Human Erythrocyte	for Biochemistry	50 $\mu$ g	2-10°C
<b>Antibodies</b>				
305-06741	Anti Ubiquitin, Monoclonal Antibody (Clone: FK1)	Nippon Bio-Test Laboratories, Inc.	1 mg	2-10°C
302-06751	Anti Ubiquitin, Monoclonal Antibody (Clone: FK2)		1 mg	2-10°C

### b. Inositol phospholipid metabolism related substances

Still more completed selection!

Inositol phospholipid is metabolized quickly, though in traces, and bears an important role in transmitting intracellular information. In this turnover, at first, phosphatidylinositol 4, 5-bisphosphate [PI (4,5) P<sub>2</sub>] is decomposed by phospholipase C activated by agonist stimulus such as hormone or nerve transmitter substance, etc. and produces two second messengers of inositol phosphatidylinositol 1, 4, 5-triphosphate (IP<sub>3</sub>) and diacylglycerol (DG). As a result, IP<sub>3</sub> gives rises to mobilization of calcium ions from endoplasmic reticulum, while DG activates protein kinase C and promotes various physiological reactions. In recent years, it has been found that PI (4,5) P<sub>2</sub> not only serves as a precursor of the second messengers but also by itself gives rise to cytoskeleton control and protein function modification, and the action of inositol phosphatidylinositols with phosphoric acid located at the third place has been attracting attention in relation to cancer.

#### ■ Inositol Phosphates <Synthesized, Assay: 98+% (NMR)>

Description		Grade	Wako Cat. #	Pkg. Size
D- <i>myo</i> -Inositol 1,4-Diphosphate Tetrapotassium Salt	[Ins(1,4)P <sub>2</sub> ]	for Biochemistry	542-01491	500 μg
D- <i>myo</i> -Inositol 4,5-Diphosphate Tetrapotassium Salt	[Ins(4,5)P <sub>2</sub> ]	for Biochemistry	545-01501	200 μg
D- <i>myo</i> -Inositol 1,3,4-Triphosphate Hexapotassium Salt	[Ins(1,3,4)P <sub>3</sub> ]	for Biochemistry	543-01781	500 μg
D- <i>l</i> -Inositol 1,4,5-Triphosphate Hexapotassium Salt	[Ins(1,4,5)P <sub>3</sub> ]	for Biochemistry	541-01461	1 mg
D- <i>myo</i> -Inositol 1,4,5-Triphosphate Tripotassium Salt	[Ins(1,4,5)P <sub>3</sub> ]	manufactured by Dojindo	347-05781	100 μg
			343-05783	1 mg
D- <i>myo</i> -Inositol 1,5,6-Triphosphate Hexapotassium Salt	[Ins(1,5,6)P <sub>3</sub> ]	for Biochemistry	540-01791	200 μg
D- <i>myo</i> -Inositol 2,4,5-Triphosphate Hexapotassium Salt	[Ins(2,4,5)P <sub>3</sub> ]	for Biochemistry	546-01771	200 μg
D- <i>myo</i> -Inositol 1,2,5,6-Tetraphosphate Octapotassium Salt	[Ins(1,2,5,6)P <sub>4</sub> ]	for Biochemistry	540-01811	200 μg
D- <i>myo</i> -Inositol 1,3,4,5-Tetraphosphate Octapotassium Salt	[Ins(1,3,4,5)P <sub>4</sub> ]	for Biochemistry	544-01451	500 μg
D- <i>myo</i> -Inositol 1,3,4,5-Tetraphosphate Tetrapotassium Salt	[Ins(1,3,4,5)P <sub>4</sub> ]	manufactured by Dojindo	349-05861	100 μg
D- <i>myo</i> -Inositol 1,3,4,6-Tetraphosphate Octapotassium Salt	[Ins(1,3,4,6)P <sub>4</sub> ]	for Biochemistry	544-01831	200 μg
D- <i>myo</i> -Inositol 1,4,5,6-Tetraphosphate Octapotassium Salt	[Ins(1,4,5,6)P <sub>4</sub> ]	for Biochemistry	543-01801	200 μg
D- <i>myo</i> -Inositol 3,4,5,6-Tetraphosphate Octapotassium Salt	[Ins(3,4,5,6)P <sub>4</sub> ]	for Biochemistry	547-01821	200 μg
D- <i>myo</i> -Inositol 1,3,4,5,6-Pentaphosphate Decapotassium Salt	[Ins(1,3,4,5,6)P <sub>5</sub> ]	for Biochemistry	541-01841	200 μg

#### ■ Phosphatidyl Inositol Phosphates <Synthesized, Assay: 98+% (NMR)>

Octanol side chain (diC8)

L- $\alpha$ -Phosphatidyl-D- <i>myo</i> -Inositol 3-Monophosphate (diC8)	[PI(3)P]	for Biochemistry	549-02361	200 μg
L- $\alpha$ -Phosphatidyl-D- <i>myo</i> -Inositol 4-Monophosphate (diC8)	[PI(4)P]	for Biochemistry	546-02371	200 μg
L- $\alpha$ -Phosphatidyl-D- <i>myo</i> -Inositol 5-Monophosphate (diC8)	[PI(5)P]	for Biochemistry	543-02381	200 μg
L- $\alpha$ -Phosphatidyl-D- <i>myo</i> -Inositol 3,4-Diphosphate (diC8)	[PI(3,4)P <sub>2</sub> ]	for Biochemistry	545-02341	200 μg
L- $\alpha$ -Phosphatidyl-D- <i>myo</i> -Inositol 3,5-Diphosphate (diC8)	[PI(3,5)P <sub>2</sub> ]	for Biochemistry	542-02351	200 μg
L- $\alpha$ -Phosphatidyl-D- <i>myo</i> -Inositol 4,5-Diphosphate (diC8)	[PI(4,5)P <sub>2</sub> ]	for Biochemistry	540-02391	500 μg
L- $\alpha$ -Phosphatidyl-D- <i>myo</i> -Inositol 3,4,5-Triphosphate (diC8)	[PI(3,4,5)P <sub>3</sub> ]	for Biochemistry	541-02321	200 μg

Palmitoyl side chain (diC16)

L- $\alpha$ -Phosphatidyl-D- <i>myo</i> -Inositol 3-Monophosphate (diC16)	[PI(3)P]	for Biochemistry	549-01881	200 μg
L- $\alpha$ -Phosphatidyl-D- <i>myo</i> -Inositol 4-Monophosphate (diC16)	[PI(4)P]	for Biochemistry	545-01481	1 mg
L- $\alpha$ -Phosphatidyl-D- <i>myo</i> -Inositol 5-Monophosphate (diC16)	[PI(5)P]	for Biochemistry	546-01891	200 μg
L- $\alpha$ -Phosphatidyl-D- <i>myo</i> -Inositol 3,4-Diphosphate (diC16)	[PI(3,4)P <sub>2</sub> ]	for Biochemistry	548-01851	200 μg
L- $\alpha$ -Phosphatidyl-D- <i>myo</i> -Inositol 3,5-Diphosphate (diC16)	[PI(3,5)P <sub>2</sub> ]	for Biochemistry	545-01861	200 μg
L- $\alpha$ -Phosphatidyl-D- <i>myo</i> -Inositol 4,5-Diphosphate (diC16)	[PI(4,5)P <sub>2</sub> ]	for Biochemistry	548-01471	500 μg
L- $\alpha$ -Phosphatidyl-D- <i>myo</i> -Inositol 3,4,5-Triphosphate (diC16)	[PI(3,4,5)P <sub>3</sub> ]	for Biochemistry	542-01871	200 μg

[Related Products]

#### ◆ Phosphatidic Acids

Description	Grade	Wako Cat. No. (Package Size)
L- $\alpha$ -Phosphatidic Acid Dimyristoyl Sodium Salt	for Biochemistry	164-16101(25 mg); 160-16103 (100 mg)
L- $\alpha$ -Phosphatidic Acid Dipalmitoyl Sodium Salt	for Biochemistry	161-16111 (25 mg); 167-16113 (100 mg)
L- $\alpha$ -Phosphatidic Acid Distearoyl Sodium Salt	for Biochemistry	168-16121 (25 mg); 164-16123 (100 mg)

#### ◆ Phosphatidylcholines

Phosphatidylcholine, from Egg Yolk	for Biochemistry	169-12751 (100 mg); 165-12753 (1 g)
L- $\alpha$ -Phosphatidylcholine, from Egg Yolk, Hydrogenated	for Biochemistry	166-15321 (250 mg); 162-15323 (1 g)
L- $\alpha$ -Phosphatidylcholine, from Soybean, Hydrogenated	for Biochemistry	163-15071 (250 mg); 169-15073 (1 g)

#### ◆ Phosphatidylethanolamines

L- $\alpha$ -Phosphatidylethanolamine Solution, from Bovine Brain	for Biochemistry	160-17301 (50 mg)
L- $\alpha$ -Phosphatidylethanolamine Solution, from Egg Yolk	for Biochemistry	163-17271 (50 mg)
L- $\alpha$ -Phosphatidylethanolamine Solution, from Soybean	for Biochemistry	166-17261 (50 mg)

#### ◆ Phosphatidylglycerols

L- $\alpha$ -Phosphatidyl-DL-glycerol, Dimyristoyl, Sodium Salt	for Biochemistry	163-15211 (25 mg); 169-15213 (100 mg)
L- $\alpha$ -Phosphatidyl-DL-glycerol, Dipalmitoyl, Sodium Salt	for Biochemistry	160-15221 (25 mg); 166-15223 (100 mg)
L- $\alpha$ -Phosphatidyl-DL-glycerol, Dipalmitoyl, Sodium Salt	for Biochemistry	167-15231 (25 mg); 163-15233 (100 mg)

#### ◆ Inositols

D- <i>chiro</i> -Inositol	for Biochemistry	090-03821 (100 mg); 096-03823 (500 mg)
<i>myo</i> -Inositol	Wako Special Grade	092-00282 (25 g); 094-00281 (100 g); 096-00285 (500 g)
scyll-Inositol	Wako Special Grade	090-04161 (250 mg); 096-04163 (1 g)

#### ◆ Phosphatidylinositols

L- $\alpha$ -Phosphatidylinositol Sodium Salt Solution, from Wheat	for Biochemistry	160-17281 (10 mg)
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#### ◆ Phosphatidylserines

L- $\alpha$ -Phosphatidyl-L-serine Solution, from Bovine Spinal Cord	for Biochemistry	167-17291 (50 mg)
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#### ◆ Kinases

Phosphoinositide 3-kinase p110 $\gamma$ , Human, recombinant, Solution	for Biochemistry	168-20531 (10 μg)
Diacylglycerol Kinase Solution from Escherichia coli	for Biochemistry	042-28041 (1 mg)

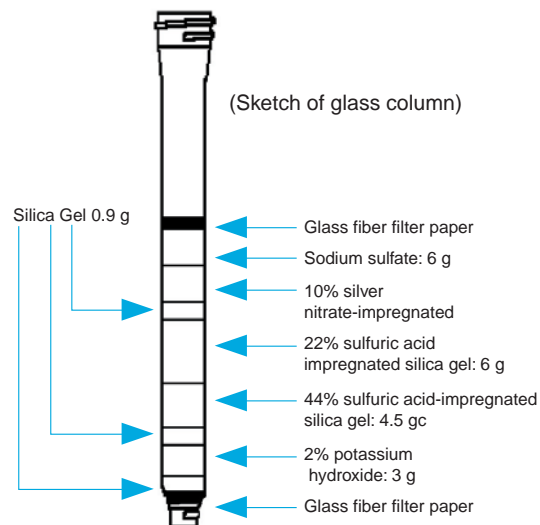
#### ◆ Lipases

Phospholipase A <sub>2</sub> , Type 1	for Biochemistry	160-20091 (1 mg)
Phospholipase D Solution	for Biochemistry	160-20111 (2,500 units)

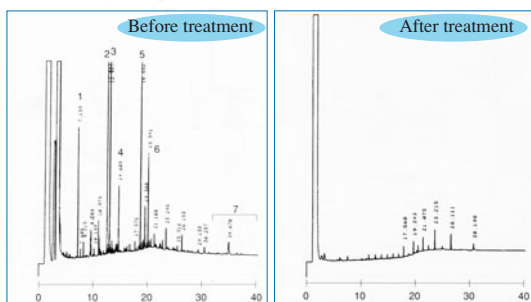
### Presep® Multilayer Silica Gel

for Dioxins Analysis  
295-41651 5 columns  
RT

As one of the pretreatment processes of dioxins analysis, clean up using a multilayer silica gel column with various kinds of chemically modified silica gel laminated is carried out in order to efficiently remove foreign substances such as compounds containing sulfur, polycyclic aromatic hydrocarbons, coloring substances, etc. which coexist in measured specimens. However, the packing operation of preparing the multilayer silica gel column to be used for this analysis is extremely troublesome. Wako has launched a product with various kinds of chemically modified silica gel (see Related Products) laminated in a glass column.



Example of clean up of soil extracted sample using Presep® Multilayer Silica Gel After treatment



1. 2,4,6-Trichlorophenol; 2. Propylamide;
3. Anthracene; 4. N,N-Bis(1-methylethyl)-benzamide
5. Lenacil; 6. Bis(2ethylhexyl)phthalate;
7. Terpenes;

#### [Features]

- \* Design that conforms to JIS-K0311 and JIS-K0312  
Column inside diameter: 15 mm  
Simplified packing procedures
- \* The practical test has been carried out.
- \* Because the product is individually packed in an aluminum package, you can use the column as many as you need.  
(Sketch of glass column)

#### [Related Products]

Cat. No.	Description	Grade	Pkg. Size
167-19251	2 % Potassium Hydroxide-impregnated Silica Gel	for Dioxins Analysis	100 g
197-11611	10 % Silver Nitrate-impregnated Silica Gel		100 g
191-11631	44 % Sulfuric Acid-impregnated Silica Gel		100 g
194-11621	22 % Sulfuric Acid-impregnated Silica Gel		100 g
194-12221	Sodium Sulfate		250 g
238-01781	Wakogel® DX		100 g

## 2. Thin Layer Chromatography

### Silicagel 70PF<sub>254</sub> Plate Wako

for Thin Layer Chromatography  
195-12871 10 pieces (20 x 20 cm)

Thin layer chromatography is an extremely simple but convenient analysis method, and is popularly used for analysis and preparation. Recently, to the existing Silicagel 70 Plate Series, new product preparative TLC plate "Silicagel 70PF<sub>254</sub> Plate Wako" was added. Porous silica gel with even size particles of pore diameter 7 nm is adopted and the amount of adhesive agent is reduced to the minimum, thereby enabling easy sampling.

The product provides excellent peeling resistance and chemical resistance against organic solvents. It is stable to sulfuric acid, iodine, ninhydrin, Dragendorff reagent, and other coloring reagents.

**NEW!**

Description	Silicagel 70PF <sub>254</sub> Plate Wako	Silicagel 70FM Plate Wako	Silicagel 70F <sub>254</sub> Plate Wako	Silicagel 70 Plate Wako
Package Size		Cat. #190-08391 10 pieces (5x10 cm)	Cat. #193-08401 10 pieces (5x10 cm)	Cat. #193-08381 10 pieces (5x10 cm)
			Cat. #193-08406 200 pieces (5x10 cm)	
		Cat. #194-08394 100 pieces (5x20 cm)	Cat. #197-08404 100 pieces (5x20 cm)	Cat. #197-08384 100 pieces (5x20 cm)
	Cat. #195-12871 10 pieces (20x20 cm)	Cat. #196-08393 25 pieces (20x20 cm)	Cat. #199-08403 25 pieces (20x20 cm)	Cat. #199-08383 25 pieces (20x20 cm)
Particle Size	5 - 40 μm	5 - 15 μm (mean: 10 nm)		
Pore Diameter	Approx. 70 Å	Approx. 70 Å		
Specific Surface Area	450 m <sup>2</sup> /g	450 m <sup>2</sup> /g		
Pore Volume	0.8 mL/g	0.8 mL/g		
Thickness of Silica Gel Layer	0.7 - 0.9 mm	230 - 250 μm		
Adhesive agent	High molecular weight polymer			
Fluorescent substance added	Single-color fluorescent substance (green) (λ = 254 nm)	Three-kind mixing fluorescent substance (red, green and blue) (λ = 250 - 400 nm)	Single-color fluorescent substance (green) (λ = 254 nm)	Not contained



Clean up of dioxins measuring samples using active carbon silica gel packed column

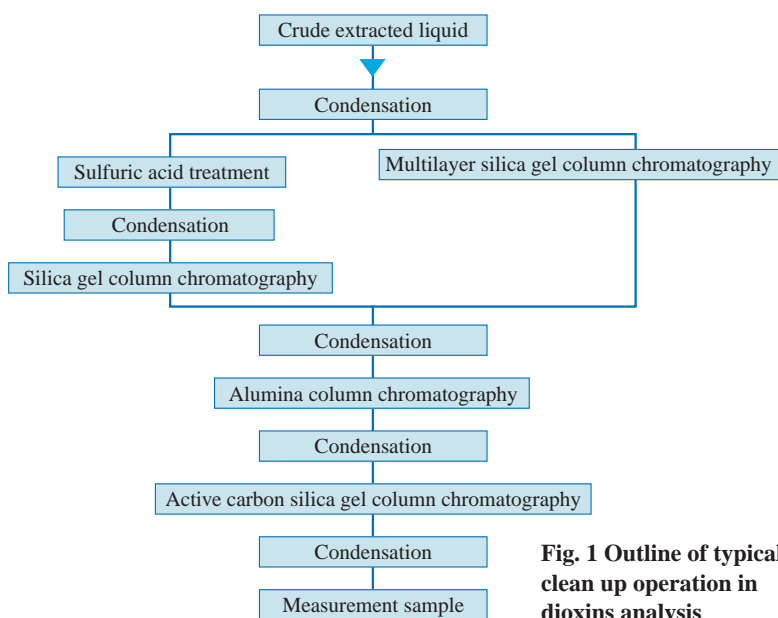
Comparison between

### Presep® Active Carbon-blended Silica Gel (Cat. #299-41551) and Presep® Active Carbon-impregnated Silica Gel (Cat. #293-41451)

- Both Active Carbon-impregnated Silica Gel and Active Carbon-blended Silica Gel have low blank values of dioxins and require no preliminary cleaning. (See Table 1.)
- Because of Active Carbon-impregnated Silica Gel, the high adsorption capacity is best suited for sample analysis that contains foreign substances such as soil, bottom sediment, etc.
- Because of Active Carbon-blended Silica Gel, the adsorption capacity lower than that of Active Carbon-impregnated Silica Gel, Active Carbon-blended Silica Gel is effective for samples that contain comparatively less foreign substances such as environmental water, blood, breast milk, etc.
- Because Active Carbon-blended Silica Gel requires only a small amount of toluene liquid to elute PCDDs/PCDFs, operating time can be shortened. (See Figure 3.)

**Table 1. Examples of reagent blank of dioxins of active carbon silica gel** (Unit: pg/g)

Dioxins	Active Carbon-impregnated Silica Gel	Active Carbon-blended Silica Gel
T4CDDs	0.2 ↓	0.2 ↓
P5CDDs	0.2 ↓	0.2 ↓
H6CDDs	0.2 ↓	0.2 ↓
H7CDDs	0.5 ↓	0.5 ↓
O8CDDs	2 ↓	2 ↓
T4CDFs	0.2 ↓	0.2 ↓
P5CDFs	0.2 ↓	0.2 ↓
H6CDFs	0.2 ↓	0.2 ↓
H7CDFs	0.5 ↓	0.5 ↓
OCDF	2 ↓	2 ↓
3,4,4'-T4CB (#81)	1 ↓	1 ↓
3,3',4,4'-T4CB (#77)	1 ↓	1 ↓
2,3',4,4'-P5CB (#118)	1 ↓	1 ↓
2,3,4,4'-P5CB (#114)	1 ↓	1 ↓
2,3,3',4,4'-P5CB (#105)	1 ↓	1 ↓
3,3',4,4'-P5CB (#126)	1 ↓	1 ↓
2',3,4,4'-P5CB (#123)	1 ↓	1 ↓
2,3',4,4',5,5'-H6CB (#167)	1 ↓	1 ↓
2,3,3',4,4'-H6CB (#156)	1 ↓	1 ↓
2,3,3',4,4',5'-H6CB (#157)	1 ↓	1 ↓
3,3',4,4',5,5'-H6CB (#169)	1 ↓	1 ↓



**Fig. 1 Outline of typical clean up operation in dioxins analysis**

#### Fractionation Performance Test

Presep® Active Carbon-blended Silica Gel

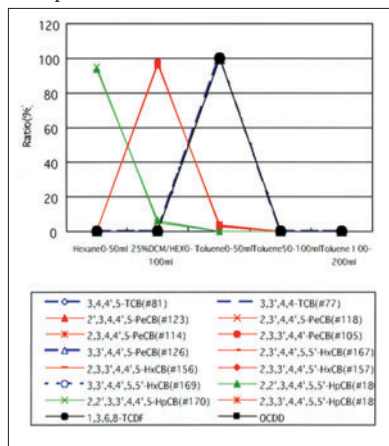


Fig. 2 Example of fractionation pattern of Active Carbon-impregnated Silica Gel column

Presep® Active Carbon-impregnated Silica Gel

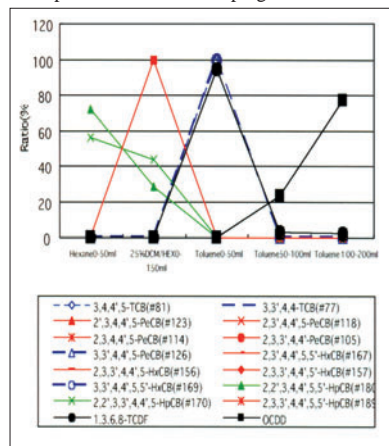


Fig. 3 Example of fractionation pattern of Active Carbon-blended Silica Gel column

(Load sample)

1,3,6,8-TCDF, OCDD 5 ng each (50 ng/mL, 0.1 mL)

Co-PCB (14 kinds) 5 ng each (50 ng/mL, 0.1 mL)

(Fractionation test conditions)

- Charge the load sample (dissolved in nonane) to the upper layer of Active Carbon-impregnated Silica Gel column or Active Carbon-blended Silica Gel column.
- After washing the glass wall surface with a small volume (200  $\mu$ L, 3 times) of hexane, allow it to stand for 15 to 30 minutes.
- Elute with the eluant.

#### [Related Products]

Wako Catalog No.	Description	Grade	Package Size
635-04191	Teflon® Cock	—	10 pieces
291-41751	Presep® Cylinder Adapter (PTFE)	—	5 pieces
297-41753			20 pieces
010-19411	Active Carbon-blended Silica Gel	for Dioxins Analysis	10 g
016-19413	Active Carbon-impregnated Silica Gel	for Dioxins Analysis	100 g
019-11941			10 g

a. Residual Chlorine Analysis

### Active Cl-DPD Test Wako (DPD (N,N-Diethyl-p-phenylenediamine Sulfate) method)

Cat. #297-56501 100 tests

### Active Cl-DPD Test Tube

Cat. #294-34151 5 tubes

#### [Features]

##### ◆ Easy operation

- No weighing is required for DPD, KI because they are supplied in tables.
- For the buffer solution, the concentrated liquid is supplied in a convenient dropper bottle.
- Measurement can be made by comparing the developed color with the color tone table.

##### ◆ Measurements are possible with one test tube.

- Both free residual chlorine and combined residual chlorine can be measured in one test tube.

#### [Kit Contents]

- |                                      |                        |
|--------------------------------------|------------------------|
| 1. DPD Tablet                        | 1 bottle x 100 tablets |
| 2. KI Tablet                         | 1 bottle x 100 tablets |
| 3. Phosphate Buffer Solution, pH 6.5 | 1 bottle x 8 mL        |
| 4. Color Tone Table                  | 1 sheet                |



Chlorine is used for sterilization and disinfection of drinking water and swimming pool water. Chlorine displays disinfecting action in the form of hydrochlorous acid in water, but reacts with suspended matters, organic matters, metallic salts, etc. in water or dissipates in the atmosphere, and its concentration lowers and disinfecting action is lost. Therefore, residual chlorine in water is measured from the viewpoint of health management.

Presently, the orthotolidine method is a mainstream of residual chlorine measurement, but carcinogenicity of orthotolidine has been pointed out and the DPD method has been attracting attention of the people concerned. The DPD method measures residual chlorine by comparing the standard colorimetric liquid to pink to pinkish red color developed by allowing residual chlorine to react with diethyl-p-phenylenediamine (DPD).

The present product is a simplified residual chlorine analysis kit with this DPD method adopted as the working principle.

#### Measuring Method

##### 1. Measurement of free residual chlorine content (Cl mg/L)

- (1) Attach the rubber lid onto the "Active Cl-DPD Test Tube and place 5 mL of test water (to the mark).<sup>Note 1</sup>

Note 1:

When the residual chlorine concentration in sample exceeds 2.0 mg/L, dilute it so that the residual chlorine concentration becomes 0.05-2.0 mg/L. In such event, find the residual chlorine concentration by multiplying the residual chlorine concentration of test water by the dilution ratio.

- (2) Add one drop of phosphate buffer.<sup>Note 2</sup>

Note 2:

Adding two drops of phosphate buffer will not cause any problems.

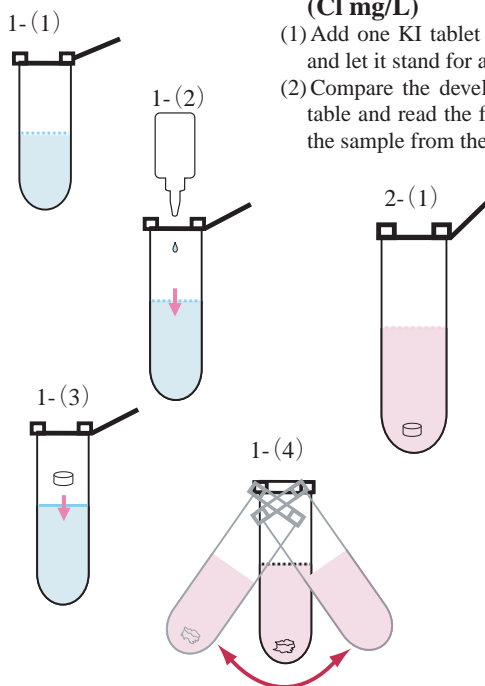
- (3) Add one DPD tablet.

- (4) Cover the test tube mouth with the lid and shake vigorously for about 5 seconds.

- (5) Compare the developed color of the sample with the color tone table and read the free residual chlorine content (Cl mg/L) (read the color of the sample from the side surface of the test tube). Allow within 1 minute or so for mixing and color development.<sup>Note 3</sup>

Note 3:

Sodium sulfate anhydride remains undissolved, but this does not exert any

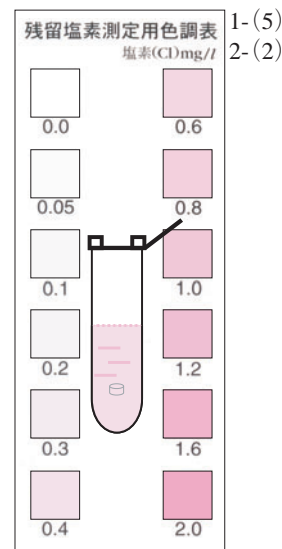


effect on the measurement. In 5 seconds, color is developed for successful analysis of the free residual chlorine content.

##### 2. Measurement of total residual chlorine content (Cl mg/L)

- (1) Add one KI tablet to the sample of Paragraph 1-(5), lightly mix, and let it stand for about 2 minutes.

- (2) Compare the developed color of the sample with the color tone table and read the free residual chlorine content (read the color of the sample from the side surface of the test tube).



### 3. Combined residual chlorine content (Cl mg/L)

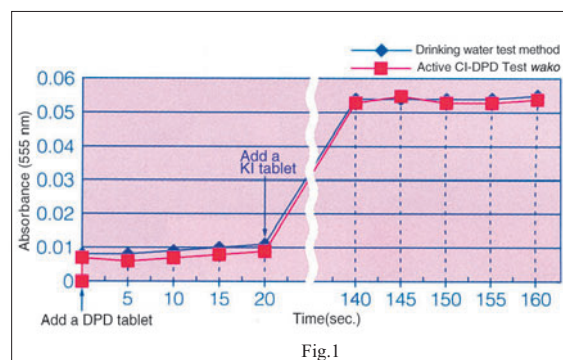
The combined residual chlorine content is the difference between total residual chlorine content and free residual chlorine content.

[combined free chlorine content (Cl mg/L)]

= [Total residual chlorine content (Cl mg/L)] - [free residual chlorine content (Cl mg/L)]

[Note] In general, the DPD method does not interfere with the following elements: Al<sup>3+</sup>: up to 4.0 mg/L; Cu<sup>2+</sup>: up to 2.0 mg/L; Fe<sup>2+</sup>: up to 3.0 mg/L; and nitrite nitrogen: up to 1.0 mg/L.

### Comparison data between the drinking water test method (conventional method) and the present kit (Fig.1)



The present kit is a simplified residual chlorine analysis kit using tablets, but the equivalent measurement data can be obtained even when the results are compared with those of the drinking water test method.

Note) Test water has nitrogen and chlorine concentration adjusted to contain a sufficient amount of combined chlorine.

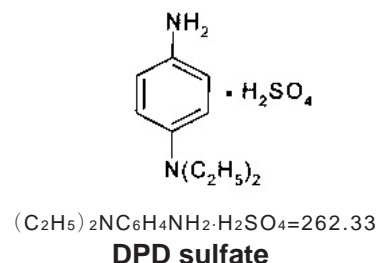
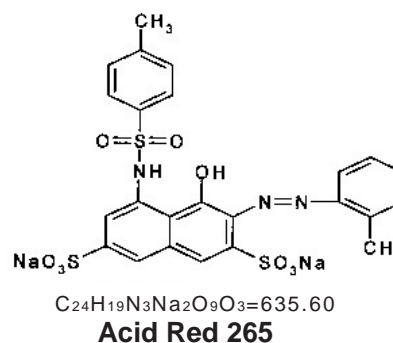
### [Product List]

Wako Cat. No.	Description	Pkg.Size
297-56501	Active Cl-DPD Test wako [Kit Contents] 1. DPD Tablet 100 tablets 2. KI Tablet 100 tablets 3. Phosphate Buffer 8 mL 4. Color Tone Table 1 sheet	100 tests
294-34151	Active Cl-DPD Test tube	5 tubes

### [Related Products]

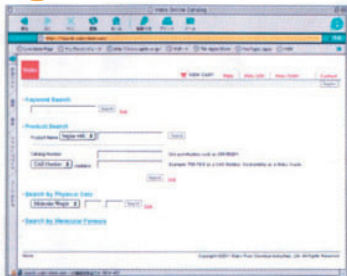
Wako Cat. No.	Description	Grade	Pkg.Size
042-27642	<i>N,N</i> -Diethyl- <i>p</i> -phenylenediamine Sulfate, 98.0+% (DPD sulfate) <Specification> Solubility in water... to pass test Suitability for pesticide residue analysis ... to pass test	for Residual Chlorine Analysis	25 g
015-18241	Acid Red 265, 98.0+% (HPLC) <Specification> Solubility in ... to pass test	for Residual Chlorine Analysis	1 g
161-20185	Phosphate Buffer Solution, pH6.5	for Residual Chlorine Analysis	500 mL
042-28002	DPD Reagent Containing DPD (4%) and (96%)		25 g
044-28001	Sodium Sulfate, Anhydrous		100 g

Reagents used for the DPD method are available. DPD sulfate is a color reagent used for DPD method, and Acid Red 265 is a pigment for adjusting standard colorimetric system.



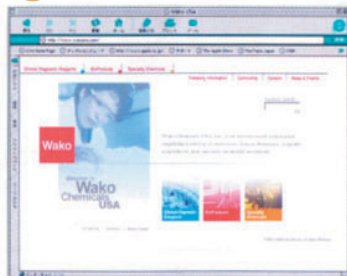
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**Wako GmbH homepage**



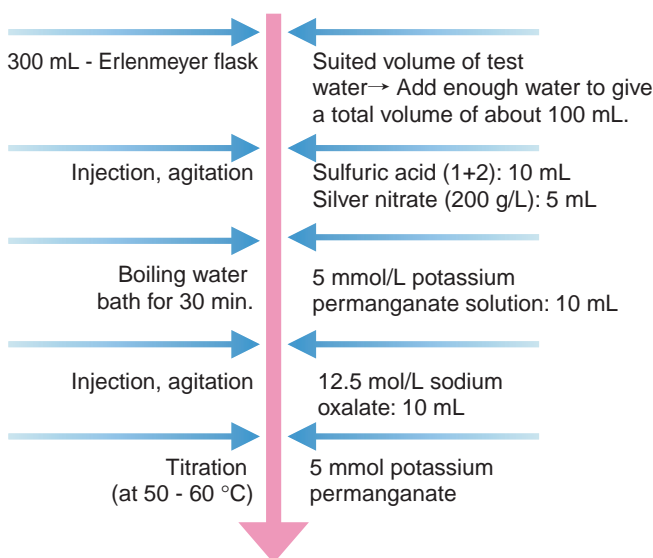
<http://www.wakochemicals.de>

## b. COD Measurement Reagents

## 20w/v% Silver Nitrate Solution

As one of the water pollutants, there are reducing substances including organic matters discharged from households and plants. A titrimetric method using the potassium permanganate solution is listed in JIS-K0101 (Testing Method for Industrial Water) and JIS-K0102 (Testing Method for Industrial Wastewater) as official methods as a method for measuring this reducing substance. Wako has a wide choice of products that meet COD analyses of these official methods.

## Analysis Flow Chart (JIS-K0101, K0102)



Wako Cat. No.	Description	Grade	Pkg. Size
161-08225	0.005mol/L Potassium Permanganate Solution (N/40)	for Volumetric Analysis	500 mL
169-08221			3 L
199-07065	0.0125 mol/L Sodium Oxalate Solution (N/40)		500 mL
197-07061			3 L
195-07067			10 L
193-08705	47 % Sulfuric Acid (1+2)	—	500 mL
197-08708			20 L
190-10702	Silver Nitrate	for COD Measurement	25 g
194-10705			500 g
<b>New!!</b> 195-12795	<b>20w/v% Silver Nitrate Solution</b>		500 mL

## c. Other

## 5w/v% Sodium Chloride Solution

for Environment Analysis

190-12821 1 L

2 - 10°C Liquid

It has been pointed out that the endocrine system of humans and wildlife may be disrupted by various environmental pollutants discharged in the environment. Researches of identifying these chemical substances and elucidating the mechanism of various toxic actions on organisms are presently underway, and specimens subject to analysis and research include river water, bottom sediment soil, foods, living organism samples, etc.

This product has impurities in the saline solution removed by the hexane extraction method and the concentrations of hormone-disrupting substances such as phthalate, alkylphenols, benzo(a)pyrene, etc. are guaranteed by the GC/MS test. You can use this product with high reliance for pretreatment of analysis of bottom sediment soil, living organism samples, etc.

## [Specification]

·Concentration (20°C)···4.5 - 5.5 w/v%

·Suitability for endocrine disrupter determination: to pass test

## [Related Products]

Wako Cat. No.	Description	Grade	Pkg. Size
018-17815	Acetone	for Environment Analysis	500 mL
015-17825	Acetonitrile		500 mL
041-28055	Dichloromethane	for Estradiol Analysis	500 mL
043-28375	Dichloromethane		500 mL
048-28065	Diethyl Ether	for Environmental Analysis	500 mL
055-06895	Ethanol		500 mL
084-07985	n -Heptane		500 mL
085-07655	n -Hexane		500 mL
135-13855	Methanol		500 mL
134-14285	Methyl Acetate		500 mL

- All products are sold for laboratory use only. They are not for use in humans.
- Please visit our online catalog to search for other products from Wako ; <http://search.wako-chem.com>
- This brochure may contain products that cannot be exported to your country due to regulations.
- Bulk quote requests for some products are welcomed. Please contact us.

037151BW

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