SUPER FRESHNESS METER KV-202

Instruction Manual

Rapid measurement of "K value", the decisive factor in freshness, is now possible. Reliable instrumentation is also available, eliminating virtually all dependence on skill or experience.

Features:

1. Can measure freshness of frozen goods.
2. Does not require special technology and measurement can be made simply.
3. Built-in micro processor provides automatic calculations, display and filing data.
4. Only a short time of 5 to 6 minutes is required for evaluation.
5. Calibration using a standard solution is unnecessary.
6. Measurement is not affected by contaminated or colored samples.

Major User:

- Fresh meat – freshness control of fish, chicken, pork, beef, etc.
- Processed food – quality control of manufacturing materials and quality evaluation of canned food.
- Electric and mechanical fields – performance evaluation of refrigerators, freezers and thawing machines.
- Research and education – development of preservation technology, study of cooking science, and as an educational tool.

CENTRAL KAGAKU CORP.

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SPECIFICATIONS

Main Body

Oxygen electrode: Polarographic membrane covered oxygen electrode

Power source: AC 100V, 50/60Hz

Dimensions: 260(W)×174(D)×148(H)mm

Weight: Approx. 2.7kg

Printer

Power source: DC 6V

Dimensions: 100(W)×150(D)×60(H)mm

Weight: Approx. 380g

Preliminary Reaction Tank

Scope of temperatures: 0 to 100℃

Power source: AC 100V

Dimensions: 130(W)×135(D)×305(H)mm

Weight: Approx. 3.4kg

Standard Accessories

Measuring reagent kit (K1 kit)	1
Extracting reagent kit	1
electrolyte (50mL)	1
electrode membrane	10
O-ring (S8×2, P8×2)	2 sets
polisher	1
micro-syringes, 25, 25, 50 μL	1 ea.
Air pump	1 set
silicone tube	2
beaker (20mL)	1
BOD bottle	2
test tube (φ10mm)	50
poly-pipette	1
stirrer bar	1

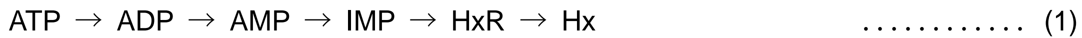
Life time of the biochemical reagents including **Measuring reagent kit (K1 kit)**

Store at cool at 4℃ (0-5℃). Do not freeze. Without contamination.

Preliminary Reacting Solution "P"	Approx. 2 months	These are enzyme reagents. Do not freeze.
Enzyme reagent "E0"	Approx. 3 months	
Buffer Solution "B"	Approx. 6 months	If these are frozen, melt at room temperature for use.
Activity Verification solution	Approx. 3 months	

WHAT IS THE K VALUE ?

After the death of a fish, the ATP contained in its muscles is decomposed sequentially by the action of enzymes which originally exist in fish muscle, as shown in formula (1). The K value is a percentage of decomposition products(HxR + Hx) to the total ATP-related compounds as shown in formula (2), which accurately reflects empirically observable changes in the freshness degree and is recognized at present as the most appropriate index of freshness.

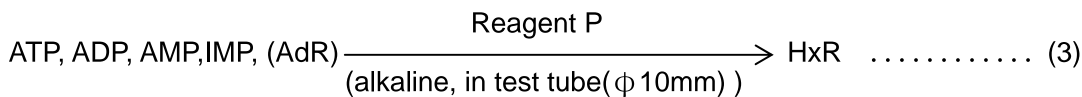


K value (%) =

$$\frac{(HxR + Hx) \times 100}{ATP + ADP + AMP + IMP + HxR + Hx} \quad \text{............ (2)}$$

PRINCIPLE

(1) Basic enzymatic reaction for determination



(2) Method of determination

1. The oxygen consumed as shown in equations (4) is measured by a Clark oxygen electrode, attached to the Reaction Cell.

2. Two moles of oxygen are consumed: one mole for each component. Therefore, each component can be determined through a dissolved oxygen depression measurement.

3. Preliminary reaction: Reagent P is used for the reaction shown as (3).

4. Reagent E0 is used for determining the K value shown as (2). 1st step reaction: for determination of HxR + Hx, and 2nd step reaction: for determination of the overall component, shown as (4).

BEFORE USING (CAUTIONS)

Do not use this machine at the place of below.
・In much humidity
・At extreme temperature
・In much dust
・On severe vibration
・Near by a large-sized electric-powered machine and a transformer
・Unstable power source

Do not spill solutions and solvents over the machine, etc.
Be careful to avoid getting the connectors wet.
Do not over-flow (from the Reaction Cell) and scatter the solutions.

The electrode is not strong, and is maybe broken easily by comparison with the other electrode. Please handle it carefully.

CAUTION! : Handling of Measuring reagent kit (K1 kit)

Measuring reagent kit contains enzyme reagents, "P" and "E0".
Be sure to store at 4℃ (0-5℃).
Do not freeze. If that is frozen at once, the activity of enzymes is decline.

When using that, to be cool that vials on ice-cooling-water, etc.
If that is warming at room temperature, the activity of enzyme is decline gradually.
Be careful to avoid freezing.
Do not have a contact with eyes, skin, cloth, etc. ("P" is alkaline)

CAUTION! : Handling of Extracting reagent kit

Measuring reagent kit contains corrosive reagents, 40% TCA and 10N KOH.
Do not have a contact with eyes, skin, cloth, etc.

TIME AND DATE SETTINGS

It is necessary to set the TIME and DATE for printing of the measuring data.

(1) Turn power switch "on" to the equipment, and "S1" LED turns on.

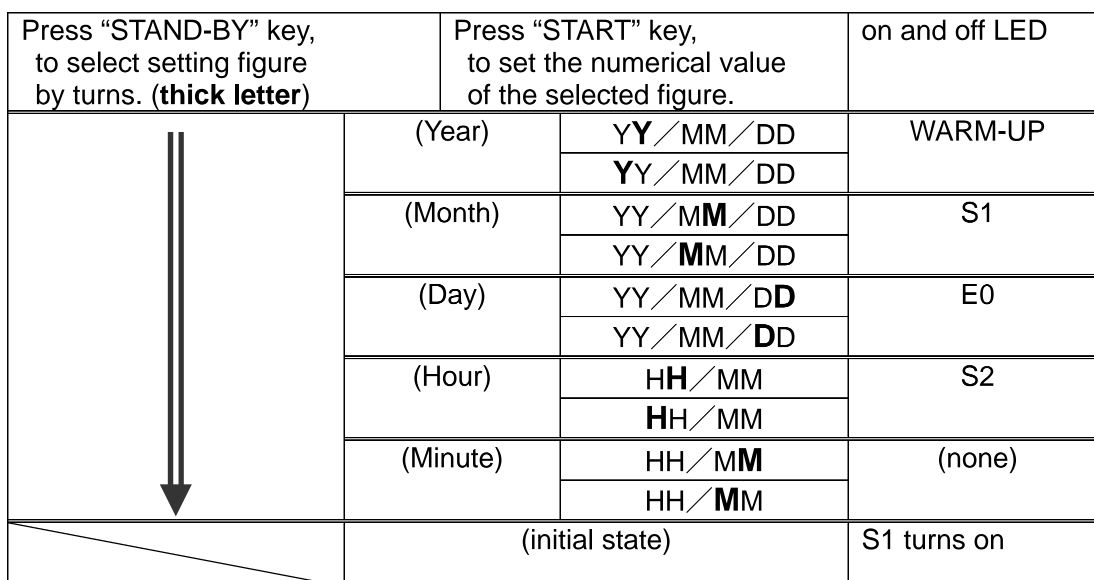
(2) Press and hold the "STAND-BY" key for approx. 3 seconds until "WARM-UP" LED goes on and off, with sound "Pi-".

(3) See this table for following procedure to set of

Y Y／M M／D D　H H／M M.

(Year) (Month) (Day)　(Hour) (Minute)

printing data :　DATE　TIME



Press "STAND-BY" key, to select setting figure by turns. (**thick letter**)	Press "START" key, to set the numerical value of the selected figure.		on and off LED
↓	(Year)	Y**Y**／MM／DD	WARM-UP
		YY／MM／DD	
	(Month)	YY／M**M**／DD	S1
		YY／**M**M／DD	
	(Day)	YY／MM／D**D**	E0
		YY／MM／**D**D	
	(Hour)	H**H**／MM	S2
		HH／MM	
	(Minute)	HH／M**M**	(none)
		HH／**M**M	
	(initial state)		S1 turns on

(4) Press "STAND-BY" key to select setting figure by turns, and turn initial state at last.

DATE　**YY／MM／DD**

SAMPLE　No.　＊

TIME　**HH／MM**

K　VALUE　＊＊．＊%

ヘンカリョウ　＊＊%

PREPARING OF THE MEASUREMENT

1. Electrode setting

◆Separate the parts of the Electrode

(1) Screw the Electrolyte Tube to remove from Cathode Holder.

(2) Remove O-ring and Separation Film Holder. Also remove Press Ring and take used Separation Film away.

◆Cleaning anode and cathode

(1) Clean(Polish) anode and cathode with Polisher, using soft cloth.

(2) After cleaning, rinse enough with distilled water.

◆Filling Electrolyte

(1) Screw the Dummy Cap on to the Electrolyte Tube.

(2) Pour the Electrolyte into the Electrolyte Tube (with dummy cap) to fill.

(3) Flip the dummy cap lightly with finger to remove bubbles from the Electrolyte.

(4) Insert the Electrode into the Electrolyte Tube with overflowing the Electrolyte, and screw on the Electrolyte tube.

◆Equipping Separation Film

(1) Remove the dummy cap. (Wipe off the drop of electrolyte that adheres to the end of the Electrolyte Tube, carefully with a paper etc. Don't to touch the cathode.)

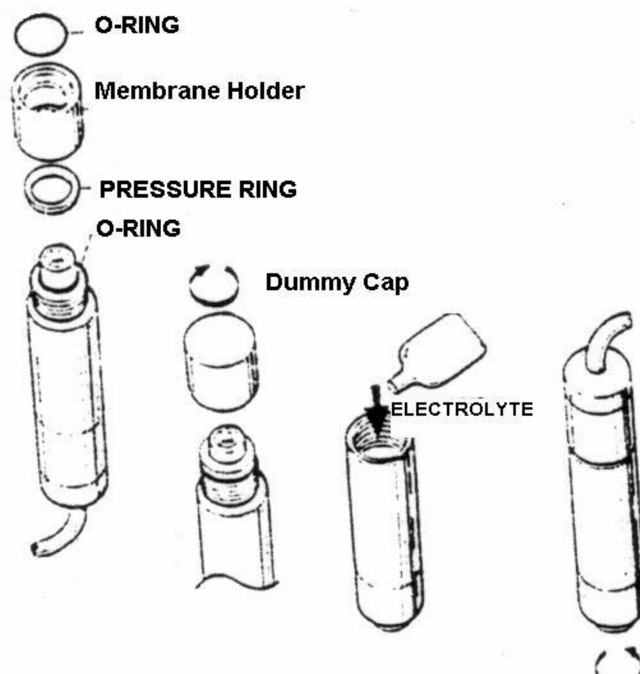
(2) Spread a suitable size (cut into about 35mm x 30mm) of Separation Film carefully over the end of electrode without any wrinkle or crumpling.

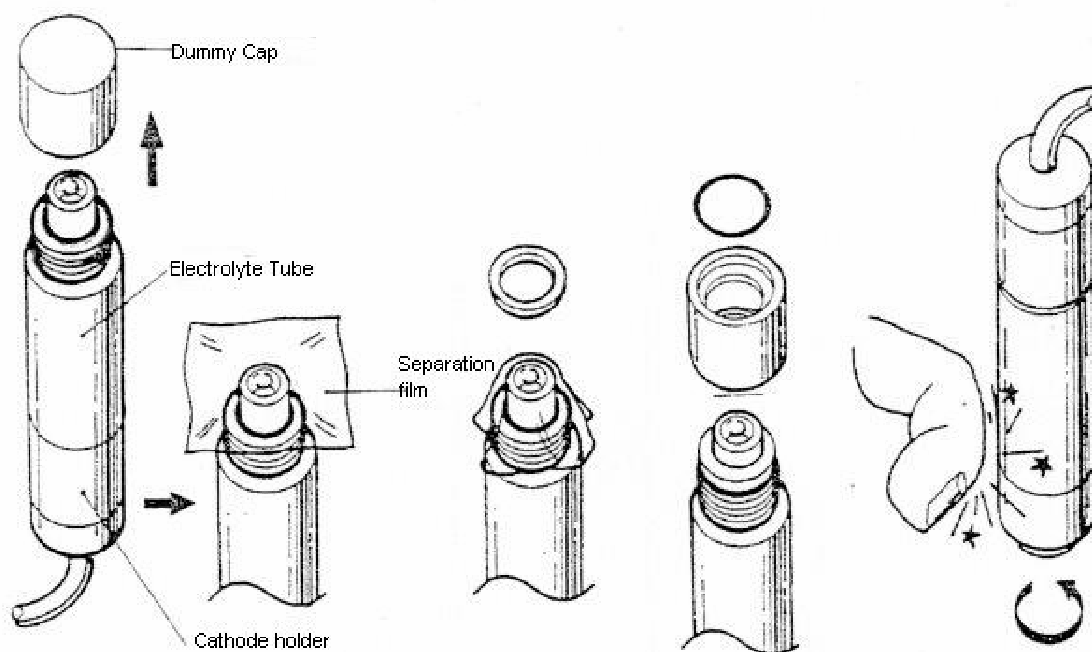
(3) Put the Press Ring to hold, and cut the extremity of the Separation Film.

(4) Screw on the Separation Film Holder tightly, and fix with the O-ring.

(5) Let the electrode end be the bottom and flipping the tube lightly to remove bubbles that adheres to the end of the cathode, screw on the holder.

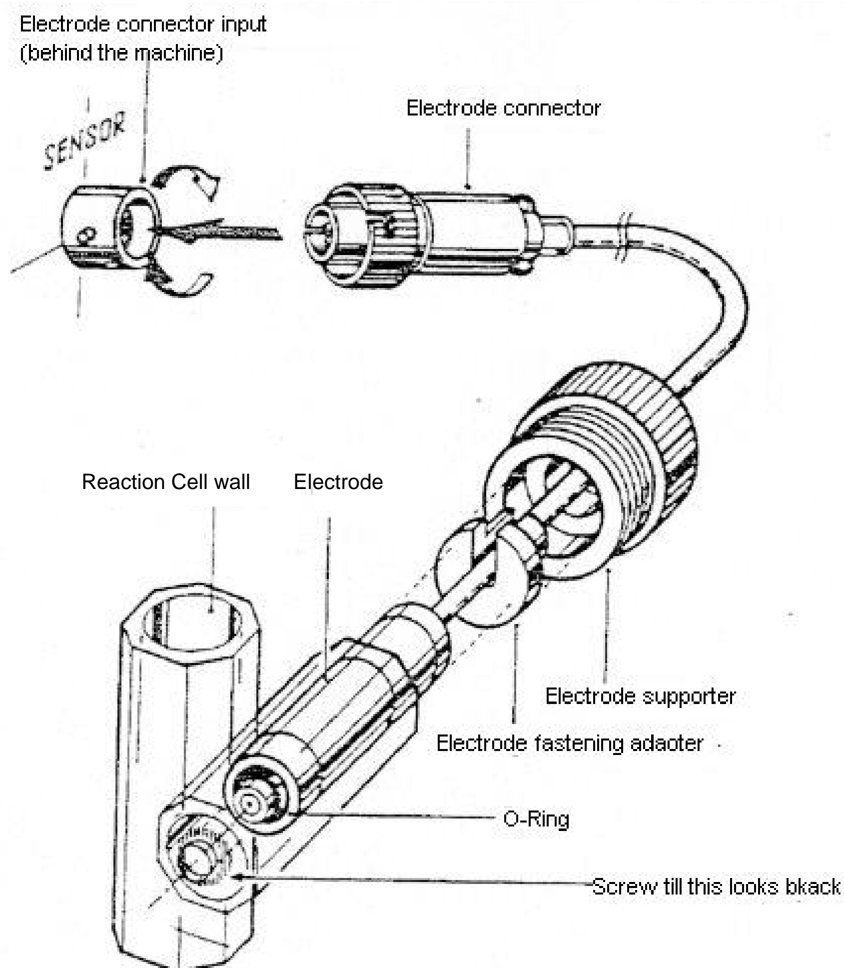
☆ PLEASE CHECK TO BE SURE THE FILM IS FREE FROM ANY WRINKLE OR BREAK.





◆Setting into the Reaction Cell

Attach the Electrode Supporter and the Electrode Fastening Adapter so as the O-ring is so tight on the Reaction Cell wall that it looks black.



◆Renewing Electrolyte and Separation Film

We recommend to replace the Electrolyte solution and the Separation Film per 1-2 weeks.

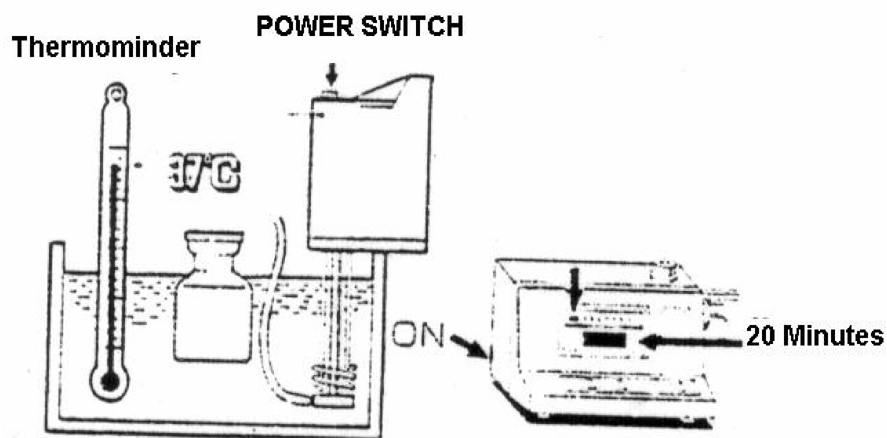
2. Warming up the instrument

(1) Fill water (pure water, drinking water, etc.) into tub. And turn power switch ”on” to each equipment.

(2) Set the thermominder temperature at 37℃.

(3) The time of warming up is 20 minutes. The time is indicated on the display.

(4) When warming up is completed, “WARM-UP” LED at guidance indicator goes off with sound “Pi-Pi-Pi”, and “S1” LED turns on.



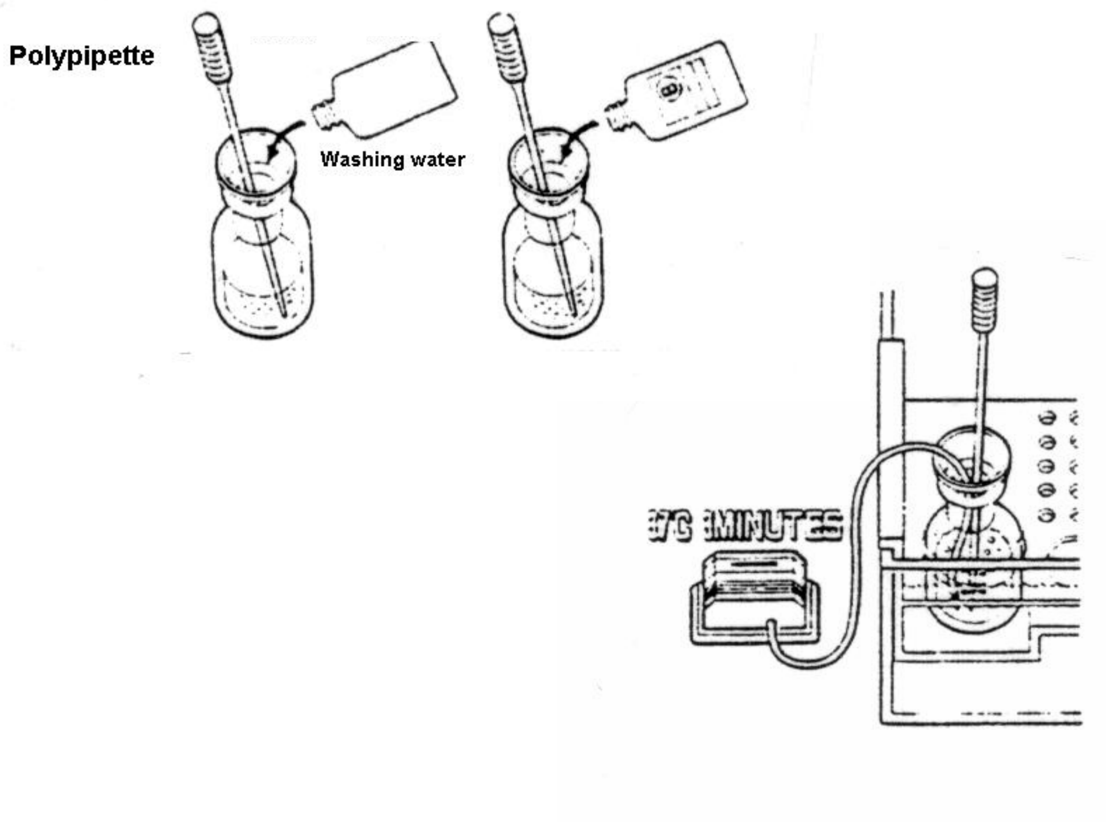
3. Preparation of the Buffer Solution

(1) Pour Buffer Solution “B” which is diluted for 2 times with distilled water and washing water (distilled water) into each BOD bottles, and put in the pipette into each.

(2) Do bubbling for 3 minutes or more by air pump, after the water temperature in the tub becomes 37℃. Diluted Buffer Solution “B” is air-saturated at 37℃.

(3) Stop bubbling before measurement, because it is necessary to remove bubbles when fill the Reaction Cell with air-saturated Buffer Solution “B”.

☆ If temperature declined, you need re-bubbling after stabilizing the water temperature.



4. Preparation of the Extracting Solution

(1) Dilute 40% TCA for 4 times with distilled water preparing 10% TCA.

(2) 10% TCA can be stored in cool (at room temperature) and dark place.

DETERMINING OF K VALUE

5. Preparation of measuring sample solution S1, S2

◆The making way of S1

(1) Weigh a cut of sample for 3-4g.

☆ In case of fish, use back meat and never use blood meat and skin.
☆ In case of meat, avoid to using grease part.

(2) Add 5ml of 10% TCA to sample and squash (crush) up.

(3) Filter the squashed sample to remove the solid.

☆ Recommendable filter paper is “No.1”.

(4) Add the one drop of Methyl Red, and naturalize it to add 10N KOH.

☆ The color of Methyl Red is changed from red to yellow, which in case of pH value is more than pH7.

☆ Please be careful to avoid over dropping.

(5) This extract is the S1 (Sample 1).

☆ Please be careful to avoid vaporizing S1.

◆The making way of S2

(1) Measure out 50μl of S1 into test tube(φ10mm) to use micro-syringe.

(2) Add 50μl of Preliminary Reacting Solution “P” into above to use micro-syringe, and mix.

(3) Seal the test tube with “PARAFILM” to avoid vaporizing.

(4) Incubate on tub at 37℃ for about 20 minutes (15minutes or more) to do preliminary reaction.

(5) After preliminary reaction, this is the S2 (sample 2).

☆ Please be careful to avoid vaporizing S2.

6. Measurement

(1) Fill the Reaction Cell with about 1ml of air-saturated Buffer Solution.

(2) Insert the Cell Cap to the Reaction Cell.

☆ Please be careful to remove bubbles in Reaction Cell.

(3) Measure out of S1, E0, S2 each with micro-syringes.
S1: 10μl, exactly
E0: 10μl
S2: 20μl, exactly

☆ Please be careful to avoid intake of the air-bubble.

(4) Inject 10μl of S1 into the Reaction Cell through the loophole of the Cell Cap.

(5) Push "START" key to start measuring.

(6) After "E0" LED turns on with sound "Pi-Pi-Pi-…", inject 10μl of E0 into the Reaction Cell through the loophole of the Cell Cap.

(7) After "S2" LED turns on with sound "Pi-Pi-Pi-…", inject 20μl of S2 into the Reaction Cell through the loophole of the Cell Cap.

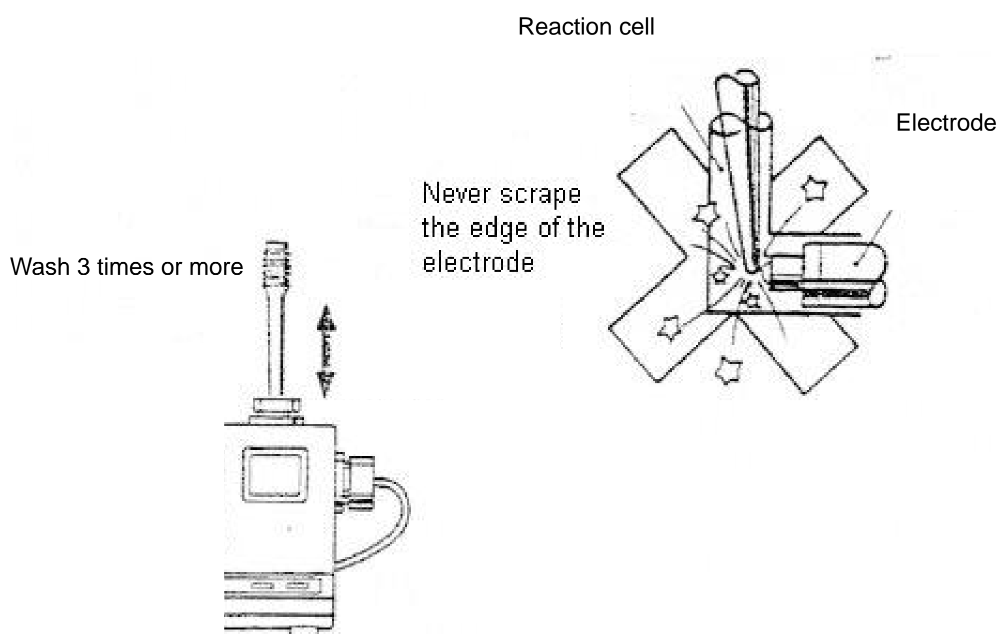
(8) With the sound "Pi-i-i-…", determined K value is indicated on the display.

(9) Push "STAND-BY" key (to print out the result with printer).

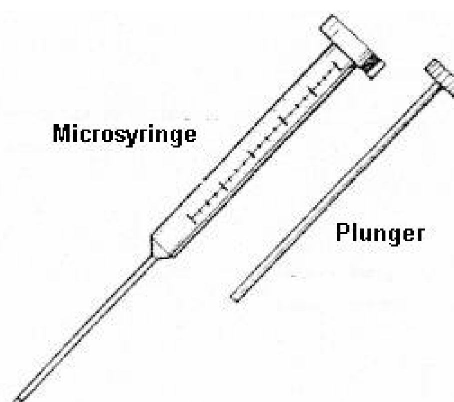
(10) Wash the Reaction Cell for 3 times or more with washing water incubated at 37℃.

(11) If push 'START' key with pushing 'STAND-BY' key, then the data history is printed out.

Please be careful to never scrape the edge of electrode.



☆ Please be careful to wash micro-syringes enough. If the plunger is not work smoothly, wash with detergent and rinse with distilled water.



7. After the measurement is done

(1) Turn off the power of the machine, the thermominder and printer.

(2) Fill distilled water in the Reaction Cell and keep it capped with the Cell Cap.

☆ Be careful not to dry the Separation Film with the Electrode. If it is not expected to use soon, keep it according to the electrode maintenance term.

(3) Keep the micro-syringes, test tube and the other after cleaning well.

TROUBLESHOOTING

●Error Code

In case the measurement is not executed accurately, an error code and the comment shall be indicated at the data indicator and printed out during the measurement or afterward.

Symptom	Meaning	Possible Remedy
E01: Output of electrode is over-range. Can not start.	Electrode is not connected. Output of electrode is over-range.	Connecting to the connector.
		Setting the electrode.
		Exchange the electrode.
	Measured sample is remained in reaction cell.	Washing the Reaction Cell.
E02: Output of electrode is not stable. Can not start.	Temperature of the Reaction Cell, the Cell Cap and diluted Buffer Solution "B" is not at 37℃.	Check temperature of tub water, and improve to at 37℃.
	Diluted Buffer Solution "B" is not air-saturated.	Do bubbling the Buffer Solution "B" for more a few minutes to be air-saturated at 37℃.
	Reaction cell is not washed enough.	Re-washing reacting cell.
	Stirrer bar is not rotate smoothly.	Exchange stirrer bar.
	Separation Film is injured.	Re-setting the electrode.
	Diluted Buffer Solution "B" is contaminated.	Re-prepare the Buffer Solution "B".
E03: Can not detect reaction of oxygen consumed	Activity of E0 is gone out.	Use new E0.
	Extract of sample is thin.	Use more weight of a cut of sample at extraction.
	Residual current is increase.	Exchange the electrode.
E04: Reaction of oxygen consumed is slow.	Activity of E0 is decline.	Use more volume of E0 (less than 30μl), or exchange E0 (use new one).
E05: Reaction of oxygen consumed is more slow (than E04).	Activity of E0 is decline.	Exchange E0 (use new one).
E06: D value (ratio of oxygen consumed) is less than 20%.	Extract of sample is thin.	Use more volume of S1 and S2 (S1 : S2 = 1 : 2, exactly), or use more weight of a cut of sample at extraction.
E07: D value (ratio of oxygen consumed) is more than 85%.	Extract of sample is dense.	Use less volume of S1 and S2 (S1 : S2 = 1 : 2, exactly), or use less weight of a cut of sample at extraction.
E08: Determined K value is more than 110%. (abnormal K value)	S1 and S2 from different extract are used.	Use S1 and S2 from same extract.
	Injected volume of S1 and/or S2 is a mistake.	Inject exactly volume of S1 and S2.
	There is some air-bubbles in the Reaction Cell.	Avoid intake of the air-bubble in the Reaction Cell.
E09: Determined K value is 0% (less than 0.04%).	S1 is not injected.	Inject S1 certainly.

●**The method of verification the Preliminary Reacting Solution "P"**

If determined K value is more than unusual or expectant value, it is possible that preliminary reaction is not successful. It is necessary to check the activity of Preliminary Reacting Solution "P", the enzyme reagent.
(If the activity of "P" is almost decline or gone out, determined K value is about 100%.)

The method is in accordance with the procedure of determining K Value of the sample, without extraction and naturalization.
Please measure "Activity Verification solution" as S1.
Expecting result of determined K value is 25-35%.
If determined K value is more, it is possible the activity of "P" is decline.

(1) Measure out 50μl of "Activity Verification solution" (as S1) into test tube(φ10mm) to use micro-syringe.

(2) Add 50μl of Preliminary Reacting Solution "P" into above to use micro-syringe, and mix.

(3) Seal the test tube with "PARAFILM" to avoid vaporizing.

(4) Incubate on tub at 37℃ for about 20 minutes (15minutes or more) to do preliminary reaction. After preliminary reaction, this is the S2.

(5) Fill the Reaction Cell with about 1ml of air-saturated Buffer Solution. Insert the Cell Cap to the Reaction Cell.

(6) Measure out of S1, E0, S2 each with micro-syringes.
S1: 10μl, exactly   E0: 10μl   S2: 20μl, exactly

(7) Inject 10μl of S1 into the Reaction Cell through the loophole of the Cell Cap. Push "START" key to start measuring.

(8) After "E0" LED turns on with sound 'Pi-Pi-Pi-…", inject 10μl of E0 into the Reaction Cell through the loophole of the Cell Cap.

(9) After "S2" LED turns on with sound 'Pi-Pi-Pi-…", inject 20μl of S2 into the Reaction Cell through the loophole of the Cell Cap.

(10) With the sound "Pi-i-i-…", determined K value is indicated on the display.