

# Rat IL12 (Interleukin 12) ELISA Kit

**Catalog Number: MBS8808164**

96 Tests

For research use only. Not intended for diagnostic use.

**Sensitivity:** 1.31 pg/mL

**Detection range:** 3.13-200 pg/mL

**Specificity:** This assay has high sensitivity and excellent specificity for detection of Rat IL12. No significant cross-reactivity or interference between Rat IL12 and analogues was observed.

**Please refer to the outer packaging label of the kit for the specific shelf life.**

## KIT components & storage

Reagents	Quantity		Storage Condition
	48T	96T	
Pre-coated Microplate	6 strips x 8 wells	12 strips x 8 wells	4°C/-20°C (6 months)
Standard (lyophilized)	1	2	4°C/-20°C (6 months)
Standard/Sample Diluent Buffer	10mL	20mL	4°C
Biotinylated Antibody (100×)	60µL	120µL	4°C/-20°C (6 months)
Biotinylated Antibody Diluent	6mL	12mL	4°C
Streptavidin-HRP (100×)	60µL	120µL	4°C/-20°C (6 months)
HRP Diluent	6mL	12mL	4°C
Wash Buffer (25×)	10mL	20mL	4°C
TMB Substrate Solution	6mL	9mL	4°C (lucifuge)
Stop reagent	3mL	6mL	4°C
Plate Covers	1	2	4°C

## Special Explanation

1. Please store the kit at 4°C if used up in one week.
2. If used for more than 1 week, store the Pre-coated Microplate, Standard, Biotinylated Antibody and Streptavidin-HRP at -20°C and all other reagents at 4°C according to the temperature indicated on the label.
3. Avoid repeated freezing and thawing.
4. Do not use the kit after the expiration date.
5. Please check whether all components are complete after opening the package.

All kit components have been formulated and quality control tested to function successfully as a kit. Do not mix or substitute reagents or materials from other kit, performance cannot be guaranteed if utilized separately or substituted.

## Materials Required, Not Supplied

1. Microplate reader capable of measuring absorbance at 450 ± 10 nm.
2. High-speed centrifuge.
3. Electro-heating standing-temperature cultivator.
4. Absorbent paper.
5. Distilled or deionized water.
6. Single or multi-channel pipettes with high precision and disposable tips.

7. Precision pipettes to deliver 2  $\mu$ L to 1 mL volumes.

## Safety notes

1. This kit is sold for lab research and development use only and not for use in humans or animals.
2. Reagents should be treated as hazardous substances and should be handled with care and disposed of properly.
3. Gloves, lab coat, and protective eyewear should always be worn, Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

## Test Principle

The test principle applied in this kit is Sandwich enzyme immunoassay. The microtiter plate provided in this kit has been pre-coated with an antibody specific to Interleukin 12(IL12). Standards or samples are added to the appropriate microtiter plate wells then with a biotin-conjugated antibody specific to Interleukin 12(IL12). Next, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After TMB substrate solution is added, only those wells that contain Interleukin 12(IL12), biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450nm  $\pm$  10nm. The concentration of Interleukin 12(IL12) in the samples is then determined by comparing the OD of the samples to the standard curve.

## Sample collection and storage

**Serum** - Use a serum separator tube and allow samples to clot for two hours at room temperature or overnight at 4°C before centrifugation for 20 minutes at approximately 1000 $\times$ g. Assay freshly prepared serum immediately or store samples in aliquot at -20°C or -80°C for later use .Avoid repeated freeze/thaw cycles

**Plasma** - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000 $\times$ g at 2-8°C within 30 minutes of collection. Remove plasma and assay immediately or store samples in aliquot at -20°C or -80°C for later use .Avoid repeated freeze/thaw cycles.

**Tissue homogenates** - The preparation of tissue homogenates will vary depending upon tissue type.

1. Tissues were rinsed in ice-cold PBS to remove excess blood thoroughly and weighed before homogenization.
2. Minced the tissues to small pieces and homogenized them in fresh lysis buffer (different lysis buffer needs to be chosen based on subcellular location of the target protein)(PBS can be used as the lysis buffer of most tissues) (w:v = 1:9, e.g. 900  $\mu$ L lysis buffer is added in 100 mg tissue sample) with a glass homogenizer on ice (Micro Tissue Grinders works, too).
3. The resulting suspension was sonicated with an ultrasonic cell disrupter till the solution is clarified.
4. Then, the homogenates were centrifuged for 5 minutes at 10000 $\times$ g. Collection the supernatant and assay immediately or aliquot and store at  $\leq$ -20°C.

**Cell Lysates** - Cells need to be lysed before assaying according to the following directions.

1. Adherent cells should be washed by cold PBS gently, and then detached with trypsin, and collected by centrifugation at 1000 $\times$ g for 5 minutes (suspension cells can be collected by centrifugation directly).
2. Wash cells three times in cold PBS.
3. Cells were then resuspended in fresh lysis buffer with concentration of 10<sup>7</sup> cells/mL. If it is necessary, the cells could be subjected to ultrasonication till the solution is clarified.
4. Centrifuge at 1500 $\times$ g for 10 minutes at 2-8°C to remove cellular debris. Assay immediately or aliquot and store at  $\leq$ -20°C.

**Urine** -Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, assay immediately or aliquot and store at  $\leq$ -20°C. Avoid repeated freeze-thaw cycles.

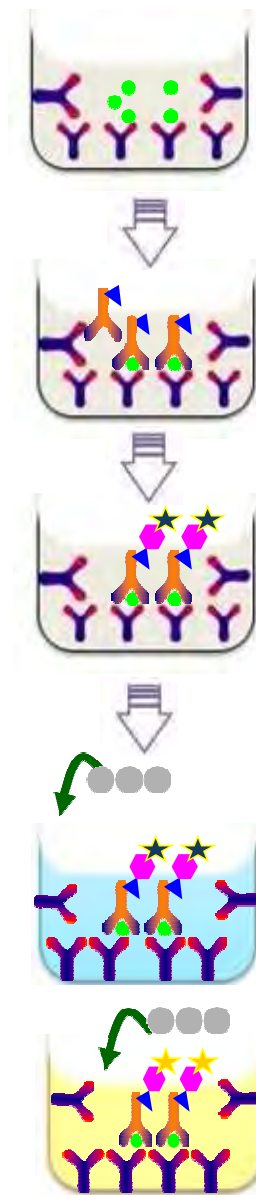
**Saliva** - Collect saliva using a collection device or equivalent. Centrifuge samples for 15 minutes at 1,000 $\times$ g at 2-8°C. Remove particulates and assay immediately or store samples in aliquot at  $\leq$ -20°C. Avoid repeated freeze/thaw cycles.

**Cell culture supernatants and other biological fluids** - Centrifuge samples for 20 minutes at 1000 $\times$ g. Collect the supernatant and assay immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles.

## Note

1. Samples to be used within 5 days may be stored at 4°C, otherwise samples must be stored at -20°C ( $\leq 1$  month) or -80°C ( $\leq 2$  months) to avoid loss of bioactivity and contamination. Avoid repeated freeze/thaw cycles.
2. Sample hemolysis will influence the result, so hemolytic specimen should not be used.
3. When performing the assay, bring samples to room temperature.
4. If the concentration of the test material in your sample is higher than that of the standard product, please make the appropriate multiple dilution according to the actual situation (it is recommended to do preliminary experiment to determine the dilution ratio).

## Summary



1. After the kit is equilibrated at room temperature, add 100 $\mu$ L of standard working Buffer (gradually diluted according to the instructions) or 100 $\mu$ L of sample to each well, incubate at 37°C for 80 minutes.

2. Discard the liquid in the plate, add 200 $\mu$ L of Wash Buffer to each well, and wash the plate 3 times. After spin-drying, add 100 $\mu$ L Biotinylated Antibody working solution to each well, incubate at 37°C for 50 minutes.

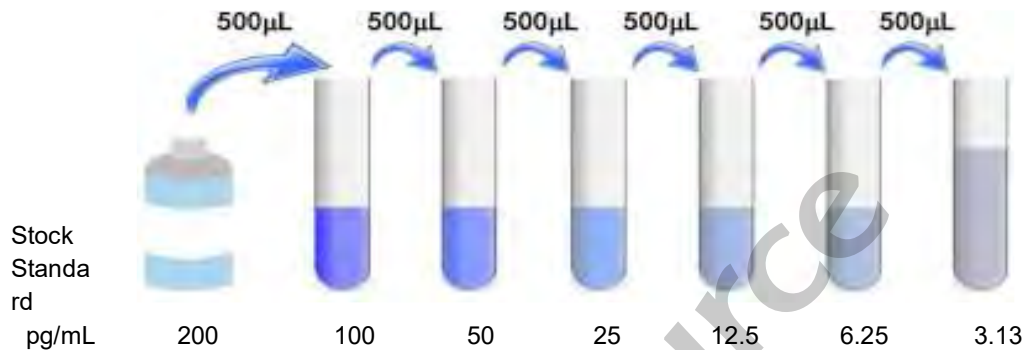
3. Discard the liquid in the plate, add 200 $\mu$ L Wash Buffer to each well, and wash the plate 3 times. After drying, add 100 $\mu$ L Streptavidin-HRP working solution to each well, incubate at 37°C for 50 minutes.

4. Discard the liquid in the plate, add 200 $\mu$ L Wash Buffer to each well, and wash the plate 5 times. After spin-drying, add 90 $\mu$ L TMB to each well, incubate at 37°C for 20min.

5. Add 50 $\mu$ L stop solution to each well, read plate at 450nm immediately, calculation of the results.

## Reagent preparation

1. Bring all kit components and samples to room temperature (18-25°C) before use.
2. If the kit will not be used up in one time, please only take out strips and reagents for present experiment, and save the remaining strips and reagents as specified.
3. Dilute the 25x wash buffer into 1x working concentration with double steaming water.
4. **Standard working solution**-Reconstitute the Standard with 1.0mL of Standard Diluent, kept for 10 minutes at room temperature, shake gently(not to foam). The concentration of the standard in the stock solution is 200 pg/mL. Please prepare 7 tubes containing 0.5mL Standard Diluent and use the diluted standard to produce a double dilution series according to the picture shown below. Mix each tube thoroughly before the next transfer. Set up 7 points of diluted standard such as 200 pg/mL, 100 pg/mL, 50 pg/mL, 25 pg/mL, 12.5 pg/mL, 6.25 pg/mL, 3.13 pg/mL, and the last EP tubes with Standard Diluent is the blank as 0 pg/mL. In order to guarantee the experimental results validity, please use the new standard solution for each experiment.



5. **Biotinylated Antibody and Streptavidin-HRP**: Briefly spin or centrifuge the stock Biotinylated Antibody and Streptavidin-HRP before use. Dilute them to the working concentration 100-fold with Biotinylated Antibody Diluent and HRP Diluent, respectively.
6. **TMB substrate** - Aspirate the needed dosage of the solution with sterilized tips and do not dump the residual solution into the vial again.

## Note

1. Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.
2. Bacterial or fungal contamination of either samples or reagents or cross-contamination between reagents may cause erroneous results.
3. All residual washing liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into the wells.
4. If crystals have formed in the Wash Solution concentrate (25x), warm to room temperature and mix gently until the crystals are completely dissolved.
5. **Prepare standards within 15 minutes before assay. This standard can only be used once.**
6. The TMB solution is light sensitive. Avoid prolonged exposure to light. Also, avoid contact of the TMB solution with metal to prevent color development. Warning TMB is toxic avoid direct contact with hands. Dispose of properly. If a dark blue color develops within a few minutes after preparation, this indicates that the TMB solution has been contaminated and must be discarded.
7. When pipetting reagents, maintain a consistent order of addition from well-to-well. This will ensure equal incubation times for all wells. Dispense the TMB solution within 15 minutes following the washing of the microtiter plate.
8. It is highly recommended to use the remaining reagents within 1 month provided this is prior to the expiration date of the kit. For the expiration date of the kit, please refer to the label on the kit box.

## Samples preparation

1. Equilibrate all materials and prepared reagents to room temperature prior to use. Prior to use, mix all reagents thoroughly taking care not to create any foam within the vials.
2. The user should calculate the possible amount of the samples used in the whole test. Please reserve

sufficient samples in advance.

3. Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

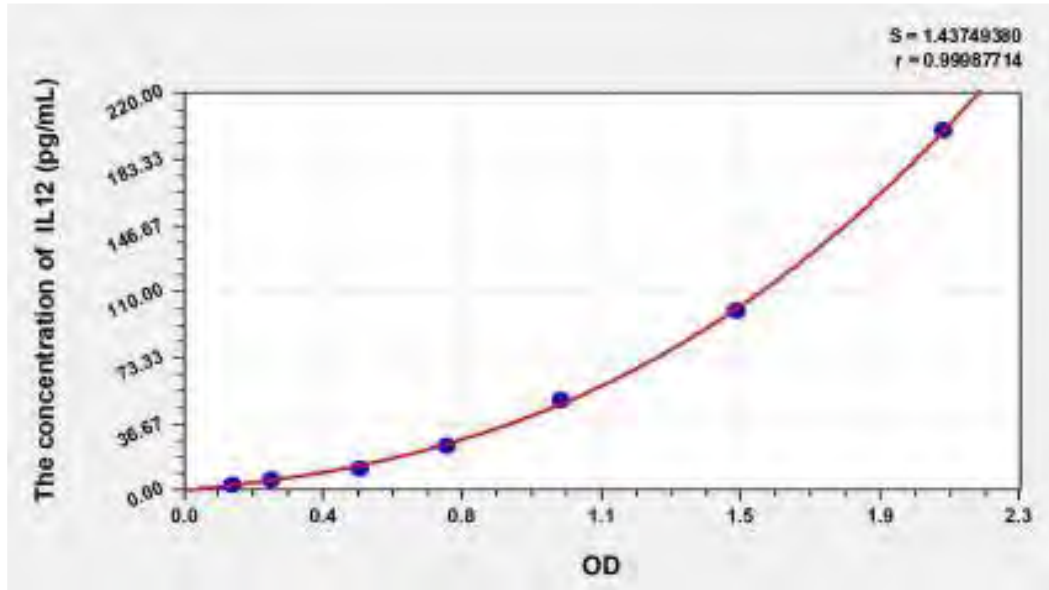
### Assay Procedure

1. Determine wells for diluted standard, blank and sample. Prepare 7 wells for standard, 1 well for blank. Add 100  $\mu$ L each of standard working solution (read Reagent Preparation), or 100  $\mu$ L of samples into the appropriate wells. Cover with the Plate sealer. Incubate for 80 minutes at 37°C.
2. Remove the liquid of each well. Aspirate the solution and wash with 200  $\mu$ L of 1 $\times$  Wash Solution to each well and let it sit for 1-2 minutes. Remove the remaining liquid from all wells completely by snapping the plate onto absorbent paper. Totally wash 3 times. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against absorbent paper.
3. Add 100  $\mu$ L of Biotinylated Antibody working solution to each well, cover the wells with the plate sealer and incubate for 50 minutes at 37°C.
4. Repeat the aspiration, wash process for total 3 times as conducted in step 2.
5. Add 100  $\mu$ L of Streptavidin-HRP working solution to each well, cover the wells with the plate sealer and incubate for 50 minutes at 37°C.
6. Repeat the aspiration, wash process for total 5 times as conducted in step 2.
7. Add 90  $\mu$ L of TMB Substrate Solution to each well. Cover with a new Plate sealer. Incubate for 20 minutes at 37°C (Don't exceed 30 minutes). Protect from light. The liquid will turn blue by the addition of TMB Substrate Solution.
8. Add 50  $\mu$ L of Stop reagent to each well. The liquid will turn yellow by the addition of Stop reagent. Mix the liquid by tapping the side of the plate. If color change does not appear uniform, gently tap the plate to ensure thorough mixing. The insertion order of the Stop reagent should be the same as that of the TMB Substrate Solution.
9. Remove any drop of water and fingerprint on the bottom of the plate and confirm there is no bubble on the surface of the liquid. Then, run the microplate reader and conduct measurement at 450 nm immediately.

### Calculation of Results

Average the duplicate readings for each standard, control, and samples and subtract the average zero standard optical density. Construct a standard curve with the Rat IL12 concentration on the y-axis and absorbance on the x-axis, and draw a best fit curve through the points on the graph. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor. Using some plot software, for instance, curve expert.

Concentration (pg/mL)	OD	Corrected OD
200	2.179	2.086
100	1.612	1.519
50	1.128	1.035
25	0.813	0.72
12.5	0.577	0.484
6.25	0.336	0.243
3.13	0.226	0.133
0	0.093	0.000



**Note: this graph is for reference only**

### Precision

Intra-assay Precision (Precision within an assay): **CV%<8%**

Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays): **CV%<10%**

Three samples of known concentration were tested in forty separate assays to assess inter-assay precision.

### Recovery

Matrices listed below were spiked with certain level of recombinant IL12 and the recovery rates were calculated by comparing the measured value to the expected amount of IL12 in samples.

Matrix	Recovery range	Average
serum(n=5)	96-107%	102%
EDTA plasma(n=5)	86-99%	92%
heparin plasma(n=5)	82-94%	88%

### Linearity

The linearity of the kit was assayed by testing samples spiked with appropriate concentration of IL12 and their serial dilutions. The results were demonstrated by the percentage of calculated concentration to the expected.

Sample	1:2	1:4	1:8	1:16
serum(n=5)	82-96%	88-101%	90-99%	87-98%
EDTA plasma(n=5)	95-102%	86-97%	88-95%	87-98%
Heparin plasma(n=5)	86-97%	90-101%	93-101%	93-102%

## Declaration

1. The kit may not be suitable for special experimental samples where the validity of the experiment itself is uncertain, such as gene knockout experiments.
2. Certain natural or recombinant proteins, including prokaryotic and eukaryotic recombinant proteins, may not be detected because they do not match the detection antibody and capture antibody used in this product.
3. This kit is not compared with similar kits from other manufacturers or products with different methods to detect the same object, so inconsistent test results cannot be ruled out.

### Analysis of common problems and causes of ELISA experiment

#### High background/non-specific staining

Description of results	Possible reason	Recommendations and Precautions
After termination, the whole plate results show a uniform yellow or light color; or the standard curve is linear but the background is too high	The yellowing of the whole plate may be caused by wrong addition of other reagents	Check the components and lot numbers of the reagents before the experiment, and confirm that all components belong to the corresponding kit. Reagents from different kits or different lot numbers cannot be mixed
	ELISA plate was not washed sufficiently	Make sure that the same amount of washing solution is added to each microwell during the washing process. After washing, press the ELISA plate firmly on the absorbent paper to remove the residual buffer.
	Incubation time too long	Please strictly follow the steps of the manual
	Streptavidin-HRP contaminates the tip and TMB container or positive control contaminates the Pre-coated Microplate	When absorbing different reagents, the tips should be replaced. When configuring different reagent components, different storage vessels should be used. Please use a pipette during operation.
	Biotinylated Antibody or Streptavidin-HRP concentration too high	Check whether the concentration calculation is correct or use after further dilution
	Substrate exposure or contamination prior to use	Store in the dark at all times before adding substrate
	Color development time is too long	Please strictly follow the steps of the manual
	The wrong filter was used	When TMB is used as the substrate, the
	when the absorbance value was read	absorbance should be read at 450 nm

### NO color plate

Description of results	Possible reason	Recommendations and Precautions
After the color development step, all wells of the ELISA plate are colorless; the positive control is not obvious	Mixed use of component reagents	Read labels clearly when preparing or using
	In the process of plate washing and sample addition, the enzyme marker is contaminated and inactivated, and loses its ability to catalyze the color developing agent..	Confirm that the container holding the ELISA plate does not contain enzyme inhibitors (such as NaN3, etc.), and confirm that the container for preparing the wash solution has been washed.
	Missing a reagent or a step	Review the manual in detail and strictly follow the operating steps

### Light color

Description of results	Possible reason	Recommendations and Precautions
The standard is normal, the color of the sample is light	The sample uses NaN3 preservative, which inhibits the reaction of the enzyme	Samples cannot use NaN3
	The sample to be tested may not contain strong positive samples, so the result may be normal	If in doubt, retest
The visual result is normal, but the reading value of the microplate reader is low	Wrong filter used for absorbance reading	When TMB is used as the substrate, the absorbance should be read at 450 nm



Description of results	Possible reason	Recommendations and Precautions
All wells, including standard and samples, are lighter in color	Insufficient incubation time	Timer accurate timing
	Insufficient color reaction	Usually 15-30min
	The number of washings increases, and the dilution ratio of the concentrated lotion does not meet the requirements	Reduce the impact of washing, dilute the concentrated lotion and washing time according to the manual, and accurately record the washing times and dosage
	Distilled water quality problem	The prepared lotion must be tested to see if the pH value is neutral.
	In the process of plate washing and sample addition, the enzyme marker is contaminated and inactivated, and loses its ability to catalyze the color developing agent.	Confirm that the container holding the ELISA plate does not contain enzyme inhibitors (such as NaN <sub>3</sub> , etc.), confirm that the container for preparing the washing solution has been washed, and confirm that the purified water for preparing the washing solution meets the requirements and is not contaminated.
	The kit has expired or been improperly stored	Please use it within the expiration and store it in accordance with the storage conditions recommended in the manual to avoid contamination.
	Reagents and samples are not equilibrated before use	All reagents and samples should be equilibrated at room temperature for about 30 minutes
	Insufficient suction volume of the pipette, too fast discharge of pipetting suction, too much liquid hanging on the inner wall of the tip or the inner wall is not clean	To calibrate the pipette, the tips should be matched, each time the tips should fit tightly, the pipetting should not be too fast, and the discharge should be complete. The inner wall of the tips should be clean, and it is best to use it once.

Description of results	Possible reason	Recommendations and Precautions
Poor repeatability	Incubation temperature constant temperature effect is not good	Keep the temperature constant to avoid the local temperature being too high or too low
	When adding liquid, too much remains on the medial wall of wells	When adding liquid, the tip should try to add liquid along the bottom of the medial wall of wells without touching the bottom of the hole.
	Reuse of consumables	The tips should be replaced when different reagents are drawn, and different storage vessels should be used when configuring different reagent components.
	The bottom of the microwell is scratched or there is dirt	Be careful when operating, be careful not to touch the bottom and wipe the bottom of the microplate to remove dirt or fingerprints
		Make 3 duplicate wells for the same sample, with 2 (including more than 2 identical results)
	Cross-contamination during sample addition	Try to avoid cross-contamination when adding samples
The color of plate is chaotic and irregular	Cross-contamination from manual plate washing	When washing the plates by hand, the first 3 injections of the lotion should be discarded immediately, and the soaking time should be set for the next few times to reduce cross-contamination.
	Cross-contamination when clapping	Use a suitable absorbent paper towel when clapping the plate, do not pat irrelevant substances into the well of the plate, and try not to pat in the same position to avoid cross-contamination

Description of results	Possible reason	Recommendations and Precautions
The color of plate is chaotic and irregular	The liquid filling head of the plate washer is blocked, resulting in unsatisfactory liquid addition or large residual amount of liquid suction, resulting in the color of plate is chaotic and irregular	Unblock the liquid addition head, so that each well is filled with washing liquid when washing the plate, and the residual amount should be small when aspirating liquid.
	Incomplete centrifugation of the sample, resulting in coagulation in the reaction well or interference of sediment or residual cellular components	Serum plasma should be fully centrifuged at 3000rpm for more than 6min
	The sample is stored for too long time, resulting in contamination.	Samples should be kept fresh or stored at low temperature to prevent contamination
	Incorrect preparation of washing solution or direct misuse of concentrated washing solution	Please configure according to the manual