

## SPHEROTEST INSTRUCTIONS

REF		PRODUCT
Sph-S02		Rabbit IgG(H+L) Spherotest Kit

### 1- KIT COMPONENTS

The available quantities of kits components are expected for 96 tests. You can choose your own standard for the calibration curve or purchase separately.

#### ➤ **PARTICLES REAGENTS**

*1 tube containing 2.2 ml of antibodies sensitized polystyrene particles, stabilized in a buffer pH 7.4. Contains bovine albumin and sodium azide 0.09 %.*

#### ➤ **DILUTION BUFFER**

*1 vial containing 58 ml of ready-to-use solution (sodium azide 0.09 %)*

#### ➤ **BLOCKING BUFFER**

*1 vial containing 15 ml of ready-to-use solution (sodium azide 0.09 %)*

**Storage:** Upon receipt, store all kit components at +2-8°C. Do **not to freeze the reagents**.

### 2- PRINCIPLE OF THE TEST

Spherotest is a **simple, rapid and specific** method for the detection of rabbit immunoglobulin using calibrated and monodispersed antibodies sensitized polystyrene microspheres.

The principle is based on micro-agglutination technique realised on standard 96 wells microplate.

Each Spherotest kit is specific for a defined species and class of immunoglobulin. It allows to quantify target antibodies in different types of samples (plasma, serum, ascite or culture supernatant) and do not cross with others proteins in sample.

#### **Technical principle:**

Single non-agglutinated submicron microspheres present a maximum of absorption at their  $\lambda_{max}$  which depends on the refractive indices of both particles and environmental buffer, and on the diameter of the particles.

Therefore, the decrease of the number of single microspheres can be optically monitored and quantified.

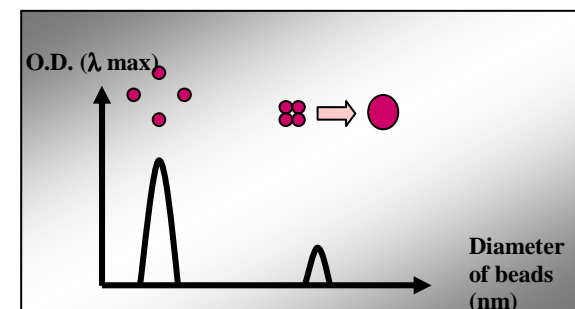
In contact with immunoglobulin, sensitized microspheres agglutinate to form clusters of bigger

apparent diameter where they do not absorb at the same  $\lambda_{max}$ .

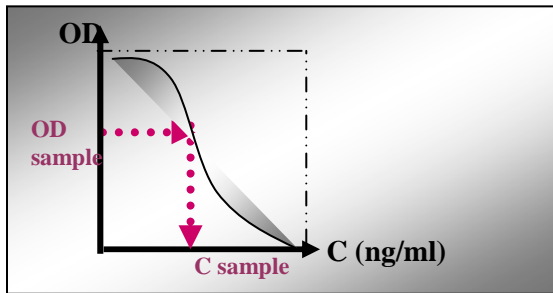
So when the antibodies sensitized beads are mixed with a sample containing immunoglobulins, they aggregate and cause a decrease adsorption of light (at 340 or 405 nm).

So low Immunoglobulin (Ig) concentrations will make high absorbance values and conversely high Ig concentrations will make low absorbance values.

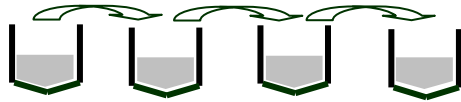
A standard curve (decrease absorbance depending of Ig concentration) can be easily obtained and allows, from absorbance response of sample, to determine Ig concentration reliably and reproducibly.



An unknown concentration of rabbit IgG can be determined on a standard curve constructed with serial dilutions of a standard sample.



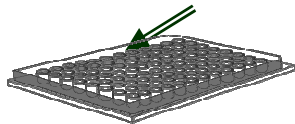
### 3- AN EASY TO USE METHOD



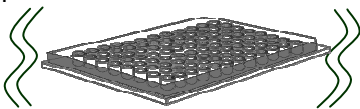
1- Prepare standard range and samples



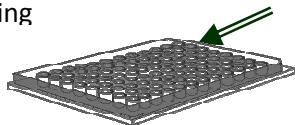
2- Suspend vigorously antibody sensitized particles



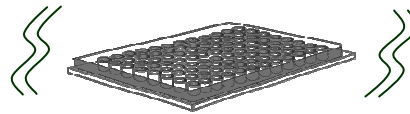
3- Add 20  $\mu$ l of the particles to wells and 20  $\mu$ l of sample



4- Incubate 5 min at room temperature with shaking



5- Add 100  $\mu$ l of Blocking buffer to each well



6- Incubate 5 min at room temperature with shaking. Measure the absorbance at 340nm (or 405 nm).

### 4- IMPORTANT RECOMMENDATIONS BEFORE USE AND ADDITIONAL NOTES

- **PRODUCT FOR RESEARCH USE ONLY.**
- **ALL REAGENTS STORE AT 2-8°C BUT SHOULD BE EQUILIBRATE AT ROOM TEMPERATURE BEFORE USE.**
- **USE ONLY REAGENTS SUPPLIED IN THE KIT AND DO NOT DILUTE THEM.**
- **PIPETTING VOLUMES AND TIMING MUST BE STRICTLY RESPECTED.**
- **FOR THE SENSITIZED PARTICLES: IT IS VERY IMPORTANT TO MIX VIGOROUSLY THE PARTICLES BEFORE PIPETING DURING AT LEAST 60 SECONDS. IT GUARANTIES THAT PARTICLES ARE CORRECTLY HOMOGENOUS AND MONODISPERSED**
- **MAKE SERIAL DILUTIONS OF SAMPLE IF YOU DO NOT KNOW CONCENTRATION LEVEL.**

- **THE WORKING RANGE OF THE SPHEROTEST IS 7.8-1000 NG/ML OF Ig.**
- **FOR PREDILUTED SAMPLES OR STANDARDS, THE FINAL CONTENT OF DEIONISED WATER SOULD NOT EXCEDED 10% OF TOTAL VOLUME.**
- **KEEP ONE WELL FREE ON THE MICROPLATE FOR THE BLANK CONTROL (EXAMPLE H12)**
- **ENSURE PREPARE A NEW STANDARD CURVE FOR EACH ASSAY ET DISTRIBUTE EACH POINT IN DUPLICATE**
- **TAKE ALLS PRECAUTIONS FOR THE HANDLING OF BIOLOGICAL SAMPLES POTENTIALLY INFECTIOUS**

### 5- MATERIALS REQUIRED BUT NOT SUPPLIED

- **Standard:** purified immunoglobulins used to calibration range. You can use your own standard or buy standard control of purified Rabbit Ig fraction.
- **Microplate:** 96-well standard transparent polystyrene microplate with flat bottom wells (we advisor to use Greiner microplate).
- **Pipettors** 10-1000  $\mu$ l.

- **Microplate shaker:** ensure the shaking speed allows to 1000 rpm. You can test the shaking speed on several assays to determine the adapted speed to apply to guaranty the reliability and reproducibility of results. Not hesitate to agitate vigorously during the first incubation for mix two drops.
- **Microplate reader** for a measure at 340 nm preferably (or 405 nm).

## 6- ASSAY PROCEDURE

- **Standard range preparation**
  - For cell culture supernatants where dilutions factors are lows, we recommend to prepare the standard range in the same matrix (culture medium) or to control matrix interferences.
  - Make first dilution (P1) of standard to 10 µg/ml using Dilution Buffer (DB).
  - Then make serials dilutions with Dilution Buffer as in table 1.

Table1: Preparation of standard range

Vial	DB Vol (µl)	STD Vol (µl)	Finale concentration (ng/ml)
PA	900	100 µl of P1	1000
PB	500	500 µl of PA	500
PC	500	500 µl of PB	250
PD	500	500 µl of PC	125
PE	500	500 µl of PD	62.5

PF	500	500 µl of PE	31.3
PG	500	500 µl of PF	15.6
PH	500	500 µl of PG	7.8

### ➤ Sample preparation

- Test different dilutions of samples to have a concentration in calibration range (7.8-1000 ng/ml). The dilution factor is depending mean concentration values in standard samples (Table 2). To be in linear yield of calibration curve, you can make serial dilutions from 1:200 to 1:1000 or dilute to a final concentration at 100-200 ng/ml.,
- Use absolutely Dilution Buffer to realise samples dilutions.

**BE CAREFUL TO USE ONLY THE DILUTION BUFFER AND NOT PBS INCOMPATIBLE WITH OUR THE ASSAY.**

Table 2: Mean concentration values in standard samples

<b>Serum/Plasma: 5-20 mg/ml</b>
<b>Ascites: 0.5-5 mg/ml</b>
<b>Cell culture supernatant: 0.01-0.05 mg/ml</b>

### ➤ Sensitized particles preparation

- Suspend the antibodies sensitized particles vigorously for at least 60 seconds just before dispensing. The base has to be dissolved and the

particles reagent must have an aspect homogenous and opaque.

### ➤ Assay protocol

- **OPERATING CONDITIONS:**
  - Spherotest assay can be realised from 20-25°C (room temperature) to 35°C without affecting the results.
  - Much as possible respected to guaranty reliable and reproducible results but incubation period between 4 and 6 min not invalid results.
  - A new Ig standard curve must be prepared for each assay as described before.
- **STEP 1: CONTACT BETWEEN COATED PARTICLES AND IG**
  - Suspend sensitized particles vigorously for 60 seconds and immediately pipette gently 20 µl. Depose on the one side of each well of a microplate.

**BE CAREFUL DO NOT LET THE PARTICLES SEDIMENT. IF THE PARTICLES VIAL IS NOT USED IMMEDIATELY, VORTEX AGAIN.**

- Calibration range: Pipette 20 µl of each calibration point and depose it in the opposite side of particles drop.

Depose each point PA to PH on each well, in duplicate to have reliable results.

- Control: Pipette 20 µl of dilution buffer (if samples are sera or plasma) or matrix to control beads dilution.

- Incubate during 5 min at room temperature with high shaking (e.g. >1000 movement/min).

**BE CAREFUL THE TWO DROPS MUST MIX WITH AGITATION. THIS STEP IS CRUCIAL TO OBTAIN RELIABLE RESULTS.**

○ **STEP 2: BLOCKING OF ASSAY**

- Add 100 µl of blocking buffer to each well (calibration point – samples and control)

- Incubate for 5 min at room temperature with moderate shaking to avoid overflow (e.g. 600 movement/min).

**BE CAREFUL THE SHAKING SPEED MUST BE SUFFICIENT. THIS STEP IS CRUCIAL TO OBTAIN RELIABLE RESULTS .**

○ **STEP 3: READING AND RESULTS**

- Measure the absorbance at 405 or 340 nm.

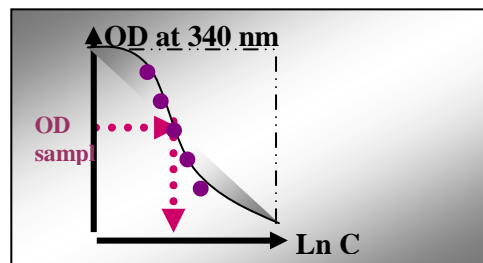
- Draw the standard curve and define the sample concentration from standard curve.

**7- RESULTS INTERPRETATION**

- The microplate reader provides absorbance for each well. The absorbance decreased in presence of Rabbit Immunoglobulins (Ig).
- The beads control absorbance has been comprised between 1.1 and 1.6.
- To draw the calibration curve, you must subtract the blank value to all raw OD (samples/control/calibration points)

$$OD = \text{raw OD} - OD \text{ blank}$$

- The calibration curve (decrease curve) allows determining samples concentrations. So low Immunoglobulin (Ig) concentrations in sample will make high absorbance values and conversely high Ig concentrations will make low absorbance values.
- E.g. Construct the standard curve OD = f(concentration) on a semi-logarithm diagram, as indicated below.



- Calculate mean of sample OD if you have made replicate and report mean value on the calibration curve. The straight linear equation defined into dynamic range can be employed to quantify Rabbit Ig.
- Calculate the sample concentration by multiplying the result by the dilution factor D.

$$C = \text{read concentration} \times D$$

**NOTE : ALL THE CALCULATIONS CAN BE PERFORMED WITH CURRENT SOFTWARES FROM YOUR MICROPLATE READER SUPPLIER.**

**Example of results**

*OD sample = 0.697*

*OD blank = 0.127*

*OD = 0.697 - 0.127 = 0.570*

*Calculated concentration = 29 ng / ml*

*Dilution of sample = 1/50 (dilution factor = 50)*

*Concentration = 29 x 50 = 1450 ng / ml = 1.45 µg/ml*

**8- PRODUCT SPECIFICATIONS**

**SPECIFICITY : RABBIT IGG (WHOLE MOLECULE)**  
**STANDARD CURVE : 7.8 TO 10000 NG/ML**  
**C.V. INTRA AND INTER-ASSAY : < 15 %**  
**TOTAL TIME OF REACTION : 10 MINUTES**  
**PARTICLE COATED ANTIBODY : ANTI-RABBIT IGG (WHOLE MOLECULE) DEVELOPED IN GOAT AFFINITY PURIFIED.NO CROSS REACTION WITH HUMAN PROTEINS.**