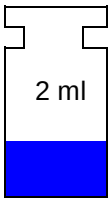
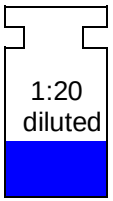
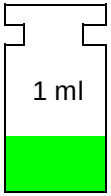
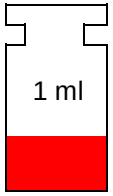
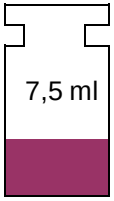
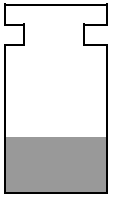
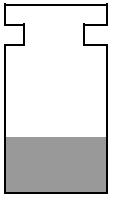
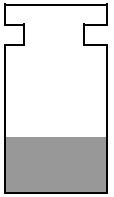
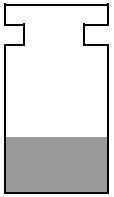
ZYMUTEST HIA (IgG or IgGAM)

HYPHEN BioMed

Package insert revision : October, 2007

RECONSTITUTION AND STABILITY OF REAGENTS WHEN RECONSTITUTED OR OPEN

Reconstituted with Distilled water		Reconstituted with HIA Sample Diluent (SD)		Reconstituted with Conjugate Diluent (CD)	Ready to Use solutions			
								
Cell lysate (CLy)	Wash Solution (WS)	Neg. Control (C-)	Pos. Control (C+)	Immunoconjugate (IC)	Sample Diluent (SD)	Conjugate Dil. (CD)	Substrate (TMB)	Stop Solution (SA)
2-8°C *2 Weeks*	*2-8°C* *7 Days*	*2-8°C* *2 Weeks*	*2-8°C* *2 Weeks*	*2-8°C* *4 Weeks*	*2-8°C* *8 Weeks*	*2-8°C* *8 Weeks*	*2-8°C* *8 Weeks*	
-20°C *2 Months*		*-20°C* *2 Months*	*-20°C* *2 Months*	*-20°C* *2 Months*				

SAMPLES AND CONTROLS

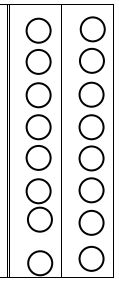
Material:	Dilution
Plasma or Serum	1:100 with HIA Sample Diluent (SD) 10 µl Sample + 990 µl HIA Sample Diluent (SD)
Neg. / Pos. Control (C- / C+)	after reconstitution: already diluted 1:100

TEST PROCEDURE

1. Step

All Wells

+ 50 µl
Cell Lysate (CLy)



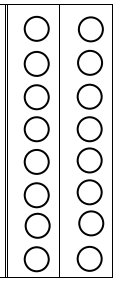
then immediately

Dilution. (SD)
+ 200 µl

Neg. Cont. (C-)
+ 200 µl

Pos. Cont. (C+)
+ 200 µl

Patient 1
+ 200 µl

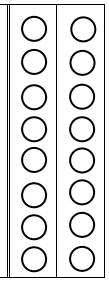


60 Minutes / 18-25°C
5 x Washing (300 µl each well)

2. Step

All Wells

+ 200 µl
Immunoconjugate (IC)

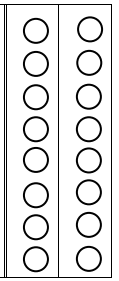


60 Minutes / 18-25°C
5 x Washing (300 µl each well)

3. Step

All Wells

+ 200 µl
Substrate (TMB)

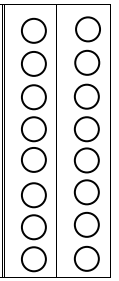


5 Minutes / 18-25°C

4. Step

All Wells

+ 50 µl
Stop Solution (SA)



after 10 – 30 Minutes
Reading absorbance at 450 nm

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QUALITY CONTROL

- Expected A450 values for positive and negative controls can present variations from lot to lot but, when the assay is run at room temperature, between 18 and 25°C, they always are:

P = A450 for positive control ≥ 1.0 N = A450 for negative control: ≤ 0.25

Obtained values for P and N, at 20±1°C, are indicated on the flyer provided in the kit.

EXPRESSION OF RESULTS

- Results are expressed according to the A450 values, as positive or negative.
- If higher dilutions are used, (i.e. D), the complementary dilution factor must be considered.

INTERPRETATION OF RESULTS

When the assay is run at 20±1°C, the results are as follows:

Positive:	A450 > 0.50
Weakly Positive:	A450 > 0.30 to < 0.50
Negative:	A450≤ 0.30

When the room temperature is out of the recommended range, absorbance values can be affected. The positive control can then be used for adjusting the cut-off value.
The flyer provided in the kit indicates the A450 value obtained for the positive control of the ZYMUTEST HIA lot used when the assay is run at **20±1°C**, and the value in % of this A450 corresponding to the cut-off.

The adjusted cut-off value is then the corresponding % of the absorbance measured for the positive control in your series of measurements.

Example for the RK040D: The flyer mentioned for the positive control an A450 at 2.10 with a % corresponding to the cut off value at 23.8% (corresponding to a cut off value at 0.50)

If in your condition, you obtained an A450 = 1.80, in your test, the corresponding cut off value is: (1.80 X 23.8) /100 = 0.428 instead of 0.50.

COMPLEMENTARY CHARACTERISATION OF POSITIVE SAMPLES (IF REQUIRED)

If required, positive samples can be further characterized by their binding inhibition in presence of heparin.
For this confirmation, to 500µl of the 1:100 diluted tested specimen, add 10µl of a 100 IU/ml Unfractionated heparin solution and mix homogeneously.
This heparinized solution (2 IU/ml final) must then be tested in the assay. Heparin dependent antibody binding to the plate is then inhibited (decrease in absorbance more than 50%) in almost all the cases. This inhibition confirms the heparin dependent binding of antibodies. In very rare specimen, already positive in the absence of platelet lysate, this inhibition is not observed, and the assay remains positive without or with heparin in the diluent: the result (which remains unclear from the present knowledge) must then be considered as inconclusive, and interpreted along with other assays or criteria for the diagnosis of HIT.