

## Product specification and information

### IRM 923 - Hev b 5 Reference Protein

1. Name of material Hev b 5 protein reference antigen
2. IRM Number/ Lot Number IRM 923/ Lot TK01-06
3. Organization preparing the material Icosagen AS (former Quattromed), Estonia
4. Antigen Specification

**Table 1**  
Specification for Hev b 5 antigen

Property	ASTM Designation	Limits/Targets
Source	D-7427, D 5900	Natural Rubber Latex C-serum
Protein amount	D-7427, D 5900	Stock solution should be adjusted to 100 ng/mL
Form		Lyophilized, white to off-white powder
RP chromatography profile	D-7427, D 5900	One major peak detectable
Mass spectrometry profile	D-7427, D 5900	One major peak detectable at molecular weight approximately 16 kDa
Capture ELISA reactivity	D-7427, D 5900	Adjust antigen content based on D-7427 determination

#### 5. Specific conditions under which the IRM is to be stored

The material is stored lyophilized in glass vials at 4°C. The shelf life of the product has not been determined but shelf life is well established for other lyophilized proteins. The reagent should have a minimum shelf life of 10 years provided that the 4°C conditions are maintained. To ensure Hev b 5 antigen quality, it should be tested for its reactivity in the IEMA assay every 24 month to ensure that degradation has not occurred.

#### 6. Specific steps before taking IRM into use

Reconstitute the lyophilized material with 565µL distilled water, mix thoroughly. As a result, the protein concentration in vial is 100 ng/mL.

Dilute reconstituted IRM solution (100 ng/mL) into reference material buffer (100mM MES, 10mM EDTA, 0.5%BPLA, 0.5% Tween20, 0.5% Dextran T-500, 0.02% Gentamycin, 0.09% NaN<sub>3</sub>, pH 6.0) in order to get at least 5 different concentrations distributed evenly over the working range of the Hev b 5 IEMA (0 ng/mL to 100 ng/mL).

Store solutions as aliquots in polypropylene tubes at -20<sup>o</sup>C, if needed. Avoid repeated freeze thaw cycles.

#### 7. Working calibrator validation using IRM

Calibrators should be prepared and used instead of reference antigen (IRM) to construct standard curves in the immunoenzymetric assay (IEMA). These calibrators must, however, meet the criteria described in D-7427 Section 8.8.1.2 or commercially-available liquid calibrators described in Section 8.8.1.1. should be used. The working calibrator must be cross-validated against its appropriate reference antigen IRM. Cross calibration (cross-validation) should be performed using the procedure provided in D-7427 Section 11.2.

#### 8. Other information

to verify the specificity and immunoreactivity of the Hev b 5 protein (IRM 923), anti-Hev b 5 capture antibody (IRM 921) and anti-Hev b 5 detection antibody (IRM 922) that are used together to perform the Hev b 5 IEMA.

