

## Product specification and information

### IRM 920 - Hev b 3 Reference Protein

1. Name of material Hev b 3 reference protein antigen
2. IRM Number/ Lot Number IRM 920/ Lot TK01-06
3. Organization preparing the material Icosagen AS (former Quattromed), Estonia.
4. Antigen Specification

**Table 1**  
Specification for Hev b 3 antigen

Property	ASTM Designation	Limits/Targets
Source	D-7427, D 5900	Field latex (FL)
Protein amount	D-7427, D 5900	Stock solution should be adjusted to 1,000 ng/mL
Form		Lyophilized, white to off-white powder
RP chromatography profile	D-7427, D 5900	One major peak detectable
Western blot profile	D-7427, D 5900	One band detectable at molecular weight approximately 20-25 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. The glass vials should be stored at 4°C. The shelf life of the Hev b 1 has not been determined, but the shelf life is well established for other lyophilized proteins. The reagent should have a minimum shelf life of 10 years provided that it is stored at 4°C. To ensure 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 542µL distilled water, mix thoroughly. As a result the protein concentration in vial is 1000 ng/mL.

Dilute reconstituted IRM solution (1000 ng/mL) into reference material buffer (100mM HEPES, 50mM NaCl, 10mM EDTA, 0.5%BPLA, 0.5% Tween20, 1% glycine, 0.5% Dextran T-500, 0.09% NaN<sub>3</sub>, pH8.0) in order to get at least 5 different concentration points distributed evenly over the working range of the Hev b 3 IEMA (0 ng/mL to 1000 ng/mL). Store solutions as aliquots in polypropylene tubes at -20°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

They also serve to verify the specificity and immunoreactivity of the Hev b 3 protein (IRM 920), anti-Hev b 3 capture antibody (IRM 918) and anti-Hev b 3 detection antibody (IRM 919) that are used together to perform the Hev b 3 IEMA.

