

## Product specification and information

### IRM 917 - Hev b 1 Reference Protein

1. Name of material Hev b 1 protein reference antigen
2. IRM Number/ Lot Number IRM 917/ Lot TK01-06
3. Organization preparing the material Icosagen AS (former Quattromed), Estonia
4. Antigen Specification

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

| Property                  | ASTM Designation | Limits/Targets  |
|---------------------------|------------------|---|
| Source                    | D-7427, D 5900   | Recombinant MBP-Hevb1   |
| 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 10-15 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 398µL distilled water, mix thoroughly. The resulting protein concentration in the vial is 1000 ng/mL or 1 microgram per milliliter.

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

To verify the specificity and immunoreactivity of the Hev b 1 protein (IRM 917), anti-Hev b 1 capture antibody (IRM 915) and anti-Hev b 1 detection antibody (IRM 916) are used together to perform the Hev b 1 IEMA.

