Species:	*Fusarium graminearum* (*Gibberella zeae*, Head Blight) and other *Fusarium* species
Product Class(es):	SBI fungicides, also suited for other fungicide classes
Method type described:	microtiter plate test
Date of protocol:	2006-05
Proven for	Tebuconazole, Prothioconazole
Should be suitable for	other SBI-Fungicides. Protocol adjustments may be needed due to the individual compound characteristics.
Version	1
comments	• validated routine method for labs equipped with microtiter plate technique • proven methodology for the active ingredients listed above. Others not mentioned have to be evaluated carefully to ensure valid results

Method:

1. Sample wheat (or other cereal) ears infected with *Fusarium* spp. (typical red color), selected at random from several plants from the field. To get an acceptable statistical result take not less than 20 ears ideally taken diagonally from the field. Don't sample immediately after rainfall. The samples should be air-dried for 24h before dispatching not to become mouldy. The ears should be transported in paper bags.

2. Desinfection of the cereal grains with NaOCl (2 %; 4 min) followed by two wash steps (sterile distilled water, 5 min each) is useful, but not absolute neccessary. Spread the infected grains in Petri dishes on potato dextrose agar (PDA, 5 grains per plate) and incubate them for 6 d at 20°C under permanent black light. Identify the developed *Fusarium* specie under the microscope

(shape/ type of conidia) and propagate the identified mycelium for purification once again for 6 d under the same conditions.

3. After 6 days of incubation at 20°C and permanent black light, the spores originating from a single colony can be harvested and used for further testing.

4. Microtiter plate test: Prepare spore suspensions in potato dextrose broth (PDB, 2500 conidia per 3 ml medium) and 96 well microtiter plates by adding 140 µl of PDB per well amended with the fungicide to be evaluated.

   Examples (final test concentrations in 200 µl):

   - Tebuconazole, Prothioconazole: 0, 0.03, 0.1, 0.3, 1.0, 3.0, 10, 30 mg/l

   The concentration range has to be adapted to a) the intrinsic activity of the fungicide to be tested and b) to the expected sensitivity variation in the fungal population.

5. Inoculate each well with 60 µl of the prepared spore suspension (50 conidia/well). Use one duplicate per isolate. Incubate microtiter plate for 4 days at 20°C, agitate gently. After incubation fungal growth is measured with a microtiter plate reader at 620 nm. Calculate EC_{50} values.

author	Andreas Mehl, Bayer CropScience AG, Alfred-Nobel-Strasse 50, 40789 Monheim, Germany Andreas.mehl@bayercropscience.com