Immunocytochemistry of fungal cells by anti-gens/species antisera using XEMA IHC anti-rabbit secondary antibody staining kit (RIG, cat№ K301)

1. Conidia and mycelium of freshly inoculated fungal culture are harvested by plastic brush from a Petri dish and washed carefully by 1M PBS

2. Mycelium suspension is centrifuged at 1500-2000g for 10 minutes; the sediment is dropped onto poly L-lysine coated glass slides and dried by air flow for 1-3 hours.

3. Dried preparations are fixed by methanol-acetone solution (1:1) for 10 minutes at 20-25°C and dried again

4. To block nonspecific binding of antibodies the sample is incubated in Blocking solution (cat№ SH001Z) from XEMA IHC anti-rabbit secondary antibody staining kit (cat№ K301) for 30 minutes in the damp chamber at 20-25°C.

5. After careful decanting of Blocking solution a working dilution of specific antiserum (antibody) is applicated onto the mycelial preparation and incubated in the damp chamber for 1 hour at 20-25°C or overnight at 4°C. After incubation, the preparation is washed by PBS 3 times for 2 minutes

6. The anti-rabbit antibodies conjugated with biotin (cat№ BH301Z) are applicated, incubated in the damp chamber for 1 hour at 20-25°C and washed from the specimen as on the previous step.

7. Streptavidin-peroxidase conjugate for IHC (cat№ TH300Z) and incubate in the damp chamber for 20 minutes at 20-25°C and washed from the specimen as on the previous step.

8. Working solution of chromogen-substrate is prepared by mixing substrate DAB concentrate (cat№ R053XZ) and DAB subsrate buffer (cat№ SR053Z) according to kit KH302 insert.

9. DAB substrate is applicated onto the specimen and incubated in the damp chamber at 20-25°C for 3-10 minutes

10. The specimen is three times washed by distilled water; “locked” by glycerol and microscopic cover glass