

Manual for GMO investigation in food samples

I. Extraction of DNA from samples

Be careful:

Reliable material: fresh material, soy flour, corn meal, corn bread mix

Not possible: oil, cereal, wheat flour

- 1.) Sign your tubes: probe (...), control (-)...
- 2.) **Fill in 500 µl InstaGene Matrix to each cup**
- 3.) Weigh out 0,5-2g of the certified non-GMO food control and also food probes
- 4.) Place each probe into a mortar
- 5.) Add 5 ml of distilled water for every gram of the probe
- 6.) Grind with pestle for at least 2 min until slurry is formed
- 7.) Add up to 5 volumes of distilled water till slurry can be transferred by pipet
- 8.) Transfer 50 µl of the slurry to the tube with Insta-Gene Matrix (s. 2)
- 9.) Shake the tube.
- 10.) Put the tube in water bath (95°C) for 5 min.
- 11.) Spin the tube in a centrifuge at max. speed for 5 min.

If tubes must be stored do so in the refrigerator.

Duplex-PCR:

GMO primer (red): Detection of 203 bp-fragment of CaMV 35S-Promoters and 225 bp-fragment of NOS terminator (85% of GMO will be detected)

Plant primer (green): Detection of 455 bp-fragment of the photosystem II as a control for good extraction.

II. PCR

- 1.) Number PCR-tubes with No. 1-6.
- 2.) Keep PCR-tubes on ice and add the following substances in numbered PCR tubes:
 - 1- 20 μ l negative control (non-GMO) and 20 μ l master-mix containing plant-primer (green)
 - 2- 20 μ l negative control (non-GMO) and 20 μ l master-mix containing GMO-primer (red)
 - 3- 20 μ l probe and 20 μ l master-mix containing plant-primer (green)
 - 4- 20 μ l probe and 20 μ l master-mix containing GMO-primer (red)
 - 5- 20 μ l positive control (GMO-DNA) und 20 μ l master-mix containing plant-primer (green)
 - 6- 20 μ l Positivkonrtolle (GMO-DNA) und 20 μ l master-mix containing GMO-primer (red)

III. Thermal cycler running conditions

Initial denaturation (one time)	2 min.	94°C	1. cycle
denaturation	1 min.	94°C	40 cycles
annealing	1 min.	59°C	„
extend	2 min.	72°C	„
extend at the end	10 min.	72°C	40. Cyclus
hold		4°C	

IV. Electrophoresis of PCR products

1. Preparation of the electrophoresis

1.1 Preparing the 1 x TAE buffer for agarose gels:

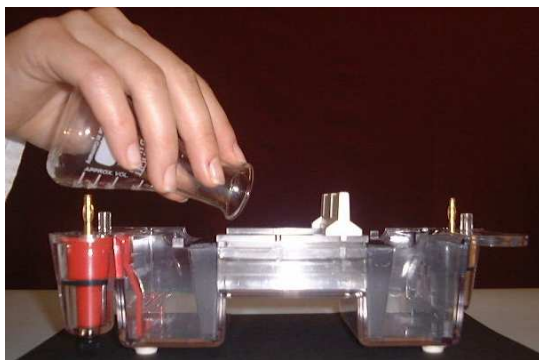
TAE stock solution must be diluted with distilled water to yield 1 x TAE buffer solution, final volume 250 ml

1.2 Preparing the 3 % (w/v) gels:

- 1,5 g agarose + 50 ml 1 x TAE buffer
- use microwave to melt agarose (avoid bubbles - do not boil!)

1.3 Casting gels:

cast gels by pouring 50 ml into the gel chamber



1.4 Preparing of the running buffer (0,25 x TAE):

dilute 125 ml 1 x TAE (s. 1.1) with 375 ml distilled H₂O

1.5 Before loading gels:

- put gels in loading position
- submerge gels with ca. 500 ml TAE buffer (0,25 x)
- remove comb right before loading the gels!

2. Preparing the probes for loading

Negative and positive controls:

- add 10 µl Orange G loading dye to each control tube (2x2)

Probes:

- add 10 µl Orange G loading dye to each probe tube

mix the tubes

Molecular weight standard (keep on ice):

- add 4 µl Orange G loading dye to 20 µl molecular weight standard and mix well.

2.2 if necessary: spin PCR tubes for 3 sec. at 2000 rpm.

3. Loading the gels:

Transfer 20 μ l of the probes, controls and molecular weight standard (orange) into the gel. If possible leave empty left and right lane.

4. Electrophoresis conditions: 200 V ca. 20 min.



run will be completed if Orange G dye front has reached 1 cm before the end of the gels.

5. Staining of DNA

- Transfer gels in carefully into staining tray.
- Submerge gels with Fast Blast (1x) and gently shaking over night.

V. Results

Compare the amplicates of your probes to the amplicates of the controls. Write down your results.

VI. References

Biotechnology ExplorerTM GMO InvestigatorTM Kit (BIO-RAD)

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