

Maize Embryo Rescue

Embryo rescue is useful when both destructive analysis of endosperm and germination of embryo are required. We routinely use the following protocol for performing gene expression and/or flow cytometric analyses on endosperms, and carrying on progenies from respective embryos. Also, mutants that show limited germination due to defective endosperms can be at times propagated this way. In our hands, 13 to 20 days-after-pollination embryos are very efficiently rescued through this procedure.

Embryo Rescue Medium

Ingredient	Final Concentration
MS Salts (Sigma's Murashige and Skoog Basal Salt Mixture)	4.33 g/L
Sucrose	20 g/L (2%)
Myoinositol	100 mg/L
Nicotinic Acid	0.4 mg/L (from 1000 x stock)
Thiamine HCl	0.2 mg/L (from 1000 x stock)
pH 5.7 - 5.8 with 1M KOH	
Agar	8 g/L (0.8%)

Melt agar into medium with a microwave oven or boiling water bath, stirring thoroughly until homogenously mixed.

Dispense 3-4 ml per 15 mm-wide, clear culture tube.

Close tubes, preferably with clear plastic caps.

Autoclave for 20 min, 120°C.

Store at 4°C in a sealed plastic bag.

Embryo Rescue Procedure

Cut off about 2 cm of ear top, so a flat surface is made.

If ear has a full set of kernels, remove a few from the central third (lengthwise) to allow access of surgical blade after sterilization, and in turn facilitate removal of kernels.

Surface-sterilize ear by immersing it on 10% (v/v) commercial bleach/distilled water for 30 min.

Rinse 3 times with sterile, distilled water.

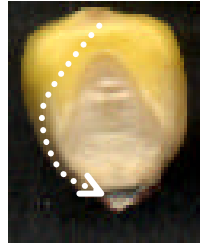
Insert a clean spatula or scalpel into the cut top of the ear for handling it.

Place base of the ear on a 150-mm diameter sterile dish, firmly holding it vertically with spatula inserted at the top.

Starting from readily accessible kernels, carefully remove them by cutting their pedicel with scalpel and no. 12 surgical blade.

Place kernels on a sterile dish, embryo side facing up.

Hold kernel gently with forceps and cut pericarp from the silk scar towards pedicel, around embryo (white arrow in figure below). If required, cut pedicel farther close to the embryo, yet avoiding the embryonic root tip.



Gently scoop embryo from below the cut pericarp with a surgical blade tip or forceps. Place embryo immediately on rescue medium.

Keep embryo in the dark at about 28°C in a growth chamber until it germinates (2-3 days). Then transfer it to an appropriate photoperiod regime (16:8 works fine for most inbreds) at 28°C.

At the 2-3 leaf stage, and provided the root system has reasonably developed, transplant seedling into appropriate soil mixture.

To prevent seedling from wilting, immediately place pot on a deep tray, keeping it covered with plastic wrap during the first days after transplanting. Gradually make holes on wrap over the following days, and finally remove it when seedling is acclimated. Seedling may then be moved to a greenhouse.