

Figure 3. Kinetics of the chemiluminescence decay in 96-well plates. The decay of the chemiluminescence signal was determined by repetitive measurement of a standard sample at 25°C with a 96-well format scintillation counter in a single-photon counting mode (TopCount).

about 20-fold higher compared to a conventional photometric quantification (under the same ELISA conditions with *o*-phenylenediamine as substrate), which reduces the use of expensive antibodies. Moreover, sophisticated detection systems like luminometers or scintillation counters for the 96-well format with capability for single-photon counting are not necessary. Thus, normal, transparent flat-bottom ELISA plates can be used, for which the individual assay was set up (irrespective of the manufacturer), and it is not necessary to switch to white or black plates (as would be for measurements with 96-well luminometers). This aspect can be very important, because significant differences can occur between the plates of different suppliers.

Another drawback of scintillation counters is that the samples are usually not measured at the same time, with the consequence that the decay curve of the chemiluminescence has to be considered. This is not true for our method, since all samples of the plate are exposed simultaneously to the film. Furthermore, it is not necessary to quantify the signal on the film with a laser scanning densitometer, since the spots

on the film show a homogeneous blackening exactly in the diameter of the wells, thus allowing a simple measurement with an ELISA reader.

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Method for Preparation of Epidermal Imprints Using Agarose

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The epidermal layer of plants carries unique and distinctive features that may serve, in most cases, as morphological markers to distinguish different plant species. These morphological features may include relative size and shape of component cells, quality and thickness of the cuticle and nature and distribution of specialized cells such as the stomata and trichomes. The venation patterns of leaves may also provide important identification points. Studies of epidermal traits gain special importance for the identification and phenotypic characterization of mutations that result in alterations of surface cells or their derivatives, and their characterization generally involves scanning electron microscopy (1).

The preparation and scanning of plant samples not only requires care and expertise but also an electron microscope. Such studies are therefore greatly curbed by the available financial and technological resources of a laboratory. Alternatively, but with a much lower degree of resolution, silicone rubber latex, collodion, cellulose acetate films, dental wax and clear lacquer/acrylic polymer have been used to obtain surface impressions, which are then recorded on photographic film for further analyses (2-4). While these substances provide good imprints, the preparation methods are also time-con-

suming and do require a certain degree of finesse and dexterity for obtaining imprints free of artifacts.

We describe a relatively simple and inexpensive technique for obtaining tis-

sue imprints that has been designed to facilitate large-scale screening of epidermal tissue in plants and for recording rapidly—induced changes in components of the epidermal layer due to

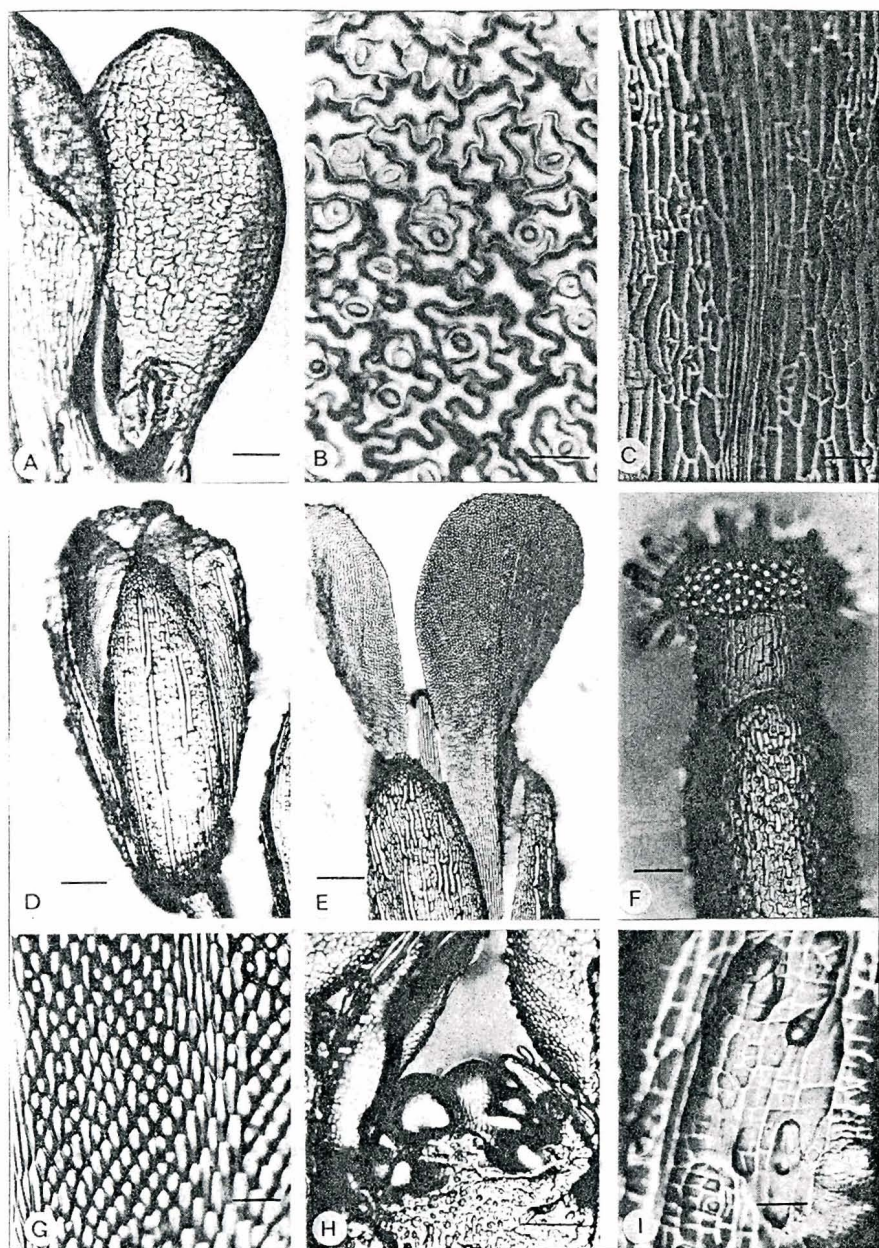


Figure 1. (A) Adaxial surface of an expanded cotyledon of *Arabidopsis thaliana* (Bar = 20 μ m). (B) Adaxial surface of a rosette leaf of *A. thaliana* showing stomata and the variable size and shape of epidermal cells (Bar = 200 μ m). (C) Epidermal surface of a developing silique of *A. thaliana* showing elongate cells interspersed with stomata (Bar = 200 μ m). (D) An unopened flower bud of *A. thaliana* showing the epidermal patterning on the sepals (Bar = 200 μ m). (E) An open flower of *A. thaliana* showing intricate cellular patterns on petals (Bar = 200 μ m). (F) The upper part of Gynoecium of *A. thaliana* (Bar = 500 μ m). (G) The abaxial surface of a leaf of a monocot plant; *Chlorophytum comosum* (Bar = 20 μ m). (H) The shoot apical meristem of *Medicago sativa* obtained by cutting longitudinally through the tip with a razor blade immediately before making the imprint. (Bar = 500 μ m). (I) And the epidermal surface of the hypocotyl of *Antirrhinum majus* 24 h after placing it on a medium containing cytokinin. The numerous cell divisions and protrusions in the epidermal cells that are clearly visible, in the imprint even at this early stage, shall become visible to the naked eye as a shoot buds only after 5–7 days (Bar = 750 μ m).

Benchmarks

exogenous applications of growth regulators or changes in other micro-environmental factors. This technique can also be extended to fresh tissue sections for studying internal anatomical details (Figure 1 H).

The method is quick to perform, and it reproduces minute cellular details (Figure 1). It utilizes the commonly available gelling agent agarose (preferably with a low melting point of around 30°–35°C such as a product from Biozym-Diagnostics, Hess, Oldendorf, Germany) and comprises the following 5 steps: (i) A 3% agarose solution is boiled in distilled water until all the agarose has melted. (ii) A thin layer is poured out on a firm, clean plastic or glass surface. (We normally use the lid of a sterile plastic petri dish.) (iii) The tissue to be imprinted is quickly laid out on the molten agarose. (Freshly cut transverse or longitudinal sections can also be used for imprinting after blot-

ting dry on filter paper for a few seconds.) (Figure 1). (iv) It is placed at 4°C for 3–5 min in a refrigerator. (The specimen may be stored in the refrigerator for longer durations up to 30 days without loss of details or resolution.) (v) The hardened agarose is removed, the tissue is peeled away and pictures are taken.

Illustrations of different tissues have been presented (Figure 1) to underline the versatility and usefulness of the method. All photographs were taken using an ordinary inverted microscope linked to a camera system (Diavert; Leitz, Wetzlar, Germany) on black and white film (Ilford PANF:ISO 50; Ilford Ltd., Mobberley, Cheshire, England, UK).

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