Step-by-step protocol for preparation of organotypic multicellular spheroids

* Collect biopsy tissue or tissue obtained by ultrasonic aspiration in the operation theatre.
* Place tissue fragments in a petri dish and wash in 5-10 ml Hanks Balanced Salt Solution with 0.9 % glucose for 30 minutes.
* Section the tissue manually with two scalpels until fragments of approximately 50 to 400 µm in diameter are obtained.
* Transfer the tumor fragments to either 0.75 % agar-coated culture flasks containing 20 ml serum-containing medium or to un-coated culture flasks containing 20 ml serum-free medium.
* Change medium the following day by tipping the culture flask in order to remove necrotic fragments. Thereafter change medium twice a week.
* After approximately 2 weeks in a standard tissue culture incubator (95% humidity, 95% air, and 5% CO2) the tissue fragments round up and form spheroids. At this time the spheroids are ready for experiments and/or fixation in 4% neutral buffered formalin for 24 hours followed by embedding in paraffin.

Composition of medium

* Serum-containing medium contain Dulbecco modified Eagle medium (D5671, Sigma-Aldrich), 10% serum (paa-A15–101, Fischer Scientific), 2% glutamine (BE17–605E, Cambrex), 4% nonessential amino acids (BE13–114E, Cambrex), and 2% penicillin/streptomycin (DE17–603E, Cambrex).
* Serum-free medium contain Neurobasal A (10888–022, Invitrogen), 0.5x B27 supplement without vitamin A (12587–010, Invitrogen), 0.5 x N2 (17502-048, Invitrogen), 1% glutamine (BE17–605E, Cambrex), 25 ng/mL EGF (E9644, Sigma-Aldrich), 25 ng/mL bFGF (100–18B, Trichem A/S), and 1% penicillin-streptomycin (DE17–603E, Cambrex). Aliquots of EGF and bFGF are added freshly before each change of medium.