

Dear customers,

Please find some basic information on polar body deposition on the AmpliGrid single cell slide in the following protocols. This manual should be seen as a rough overview on single cell handling. Please compare it with your established protocol. If there are major differences please contact the Advalytix support. In case of any other questions please do not hesitate to contact us.

Your Advalytix service team

Deposition of cells / polar bodies

- Tear open the aluminium pouch of the single cell slide.
- Take out the slide without touching the surface
- Place slide on a clean and dust free surface. Ensure that the engraved markings are readable
- Prepare 3 aliquots of this solutions in a petri dish:
 - Standard medium
 - Standard medium
 - Ammonium buffer (1x conc, ABgene, Cat.# AB-0356) or 0.5x PBS
- Cover the droplets in the pertri dish with oil
- Extract th epolar body from the oocyte in the first buffer. (transfer oocyte back to the culture vessel.)
- Transfer polar body to the second medium droplet.
- Wash the polar body shortly.
- **Optional:** To control polar body deposition it is possible to stain the cells. Please refer to the protocol on the next page for further information
- Transfer the polar body to the ammonium buffer and wash shortly..
- Deposit the polar body with e.g. a capillary to the centre of on eof the circles on the single cell slide. Please keep the liquid volume transferred as small as possible (0.1 – 0.5 µl). Inhibition of PCR might occur when transferred to high volumes due to increased salt concentration.
- Document the deposition in the form attached to the slide (Remarks, e.g. fragmented, bursted, age of cell,etc.).
- Let air dry the liquid.
- Do NOT carry out subsequent steps like e.g. fixation.
- Pack the single cell slide in the plastic box and the plastic bag.
- Please refer to the document „Single cell Service“ for further processing of your order.

Polar body / single cell staining

Material:

- Hoechst-Dye **Bisbenzimid H 33342** (Fluka, Cat.#14533), store at +4°C - +8°C (dark!)
- PCR clean water(Sigma, Cat.#W1704)

Preparation Hoechst-Dye stock solution100x:

- Dilute 1 mg Hoechst-dye in 1 ml PCR clean water
- Expiry: 1 month, light protected at +4-+8°C

Preparation Hoechst-Dye working solution 1x:

- Dilute Hoechst-Dye stock solution 100x to 1x with buffer used for polar body deposition.
- Example: 0.5µL Hoechst-Dye stock sol. 100x + 49.5 µL ammonium buffer or 0.5x PBS

Staining protocol:

- Incubate polar body for 5-30 min in Hoechst Dye working solution.
- Deposit polar body on AmpliGrid without further processing. The dye does not inhibit subsequent reactions.