

Technical Bulletin #1-98

SUBJECT: AVOIDANCE OF ELISA KIT REAGENT CONTAMINATION

The *Cygnus Technologies* ELISA Kits for detection of bioprocess impurities are very sensitive methodologies capable of detecting analytes in the pg/mL to ng/mL range. Some of these analytes such as human and bovine HCPs, BSA, HSA, Immunoglobulins and Transferrin may be found in the laboratory in very concentrated forms such as cell culture media, upstream product purification samples, human and animal sera, other laboratory buffer reagents, or from the lab technician. These materials can contain on the order of mg/mL or several million fold higher than the limit of detection of the impurity assay. These concentrated sources of the analytes can contaminate work surfaces and pipettes or otherwise become airborne by way of aerosolization. Even parts per million contamination of the air or equipment could result in very significant contamination of the ELISA kit reagents.

For example, airborne contamination of the microtiter strips used in our kits will typically manifest itself as poor duplicate precision with the inappropriate value being high. Similarly, contamination of one of the liquid reagents such as the antibody conjugate will result in high background absorbances and thus reduced assay sensitivity even though precision may appear acceptable. To minimize these problems and insure optimum performance of the assay we recommend the following precautions:

- Do not perform the assay in laboratories where concentrated forms of the analytes have been utilized such as where cell culture media or sera are present.
- Clean all work surfaces and equipment before performing the assay to reduce dust and other airborne particles.
- Dander or mucosal aerosols from the laboratory technician performing the assay can be a significant source of contamination in human cell line based HCP assays such as PER.C6®, HEK 293, A549, or Vero cell and in assays for human proteins such as HSA, human transferrin, or human immunoglobulins. Do not talk or breathe over an uncovered microtiter plate. Consider pipetting reagents in laminar flow barrier hood. Use of gloves and face mask will minimize contamination.
- Avoid use of pipettes previously used to dispense concentrated forms of the analyte.
- If possible, use disposable pipette tips with aerosol barrier filters as are employed in molecular biology procedures such as PCR.
- Do not use automated plate washers that have been subjected to concentrated solutions of the analytes, i.e. many ELISA assays already in use in your laboratory will employ a wash buffer containing BSA or various animal sera to block and wash off non-specific binding. Even after extensive flushing of this equipment with water, significant contamination will remain.
- The PNPP substrate used in some kits can be easily contaminated with environmental sources of phosphatase enzymes such as from airborne bacteria or human dander or mucosal aerosols. To minimize this source contamination, only withdraw as much substrate from the bottle as is needed for the particular assay run. Recap the substrate vial and return to the refrigerator. Do not return unused substrate back to the substrate bottle.
- After adding reagents to the wells, place the microtiter strips into a zip-lock plastic bag to protect from airborne contamination during incubation steps. We do not recommend the use of adhesive backed plate sealing tape for covering the wells as these can often introduce assay variability. Tightly recap all reagent bottles immediately after use.

Company Information

To obtain additional product information contact Cygnus Technologies:

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