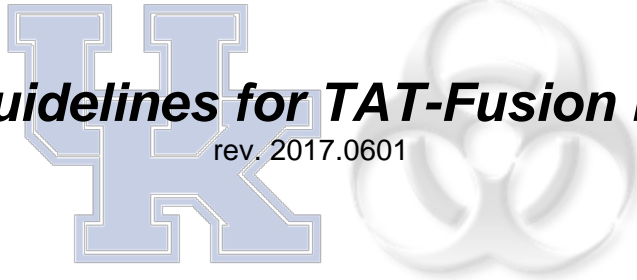


Minimum Guidelines for TAT-Fusion Protein Use

rev. 2017.0601



Background Information

The Institutional Biosafety Committee (IBC) has recently become aware of investigators either wanting to purchase/use Trans-Activating Transduction (TAT)-fusion proteins or other tags which promote protein entry into cells. Many investigators initially view TAT- fusion protein expression vectors as; just one of the many plasmids, which they may use in their laboratory and as such, submission to the IBC may be a surprising requirement. They may also view use of TAT-fusion proteins outside the purview of the IBC. Expression of a TAT- fusion protein, even in bacteria, is considered rDNA work and falls under the auspice of the IBC particularly since the TAT-fusion protein has potentially distinctive and unknown infectious qualities. As such, the use of the TAT-fusion protein is categorized as biosafety level 2 (BSL2).

TAT-fusion protein research must be approved by the IBC prior to its initiation. When any revision to an approved protocol is desired, an amendment must be filed with the IBC. The IBC reserves the right to approve exceptions to the above guidelines on a case-by-case basis. A protocol or an amendment to an existing protocol must be submitted to purchase, synthesize or express TAT proteins.

The protocol or amendment must indicate:

- Peptide to which you are linking.
- Target cells you are using.
- What are the harmful consequences, if any, when expressed?
- What are the harmful consequences, if any, in the event that personnel are inadvertently exposed?

The use of TAT-fusion proteins may be pursued at the University of Kentucky if the procedure detailed below is followed.

Procedure

- Containment of TAT-fusion protein: Executed using (BSL-2) practices
 - Laboratory Containment, Practice and Technique
 - TAT-fusion protein must be handled as a potentially hazardous material.
 - Some proteins are more toxic and/or immunogenic and should be identified.
 - Plastic backed absorbent lab paper should be used on all laboratory bench surfaces to absorb spills and splashes. All things that come in contact with TAT-fusion proteins should be regarded as contaminated.
 - Biological safety cabinet (preferred) or designated space is recommended.

- Avoid aerosol-generating activities or use appropriate safety equipment such as biological safety cabinets and sealed centrifuge tubes.
 - Personal Protective Equipment (PPE)
 - Mouth Pipetting is NOT allowed.
 - Lab coats or gowns; closed front
 - Disposable latex, nitrile, or equivalent gloves
 - Safety goggles
 - Avoid direct contact with the skin, cuts or mucous membranes.
 - Wash hands well after working with TAT material.
- Decontamination Procedures:
 - In the event of a spill while wearing gloves, lab coat or gown, and safety goggles:
 - Decontaminate work surfaces using a detergent with a protease enzyme (i.e. Terg-A-Zyme) for a minimum of 20 minutes.
 - Proceed to wash with water and then wipe with 70% ethyl alcohol solution.
- Disposal Procedures:
 - Deactivate and dispose of TAT solutions and cultures using standard autoclave methods.
 - Dilute solutions can be deactivated using a 1:10 dilution of bleach (sodium hypochlorite solution) in a 1:1 mixture with the TAT solution.
 - Allow to stand for 5-10 minutes.
 - Dispose of solution down the sewer drain with copious amounts of water.

References

Becker-Hapak M, McAllister SS, Dowdy SF. "TAT-mediated protein transduction into mammalian cells". *Methods*. 2001 Jul; 24(3):247-56. Review.

Schwarze SR, Hruska KA, Dowdy SF. "Protein transduction: unrestricted delivery into all cells?" *Trends Cell Biol*. 2000 Jul; 10(7):290-5. Review.

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