## APEX2

APEX2 is an improved version of APEX developed by the Ting laboratory<sup>1</sup>. Features:

- Improved activity over APEX and W41FAPX (dimeric) in every mammalian cell compartment we have tested
- Superior for proteomics applications with biotin-phenol<sup>2</sup>
- Superior for EM imaging with DAB<sup>3</sup>
- Monomeric and 28 kD
- Incorporates one additional mutation relative to soybean APEX<sup>2</sup>: A134P

## Our recommendation:

- Use APEX2 over APEX for proteomics<sup>2</sup> and EM<sup>3</sup> applications
- Use APEX2 over W41FAPX (dimeric) for proteomics2 and EM3 applications
- If you have already started using APEX and it works fine, then continue using it
- If you have already started using APEX and have problems of low activity, then
  try APEX2 instead and you should see a significant improvement. Note that
  APEX2 is not expected to solve other problems, such as those related to
  expression or trafficking, since it is the same size as APEX and has similar
  stability.

Summary of APEX-related constructs available on addgene:

- APEX2:
  - K14D/W41F/E112K/A134P quadruple mutant of soybean ascorbate peroxidase
  - o Addgene construct numbers 49386 and 49385
- APEX:
  - o K14D/W41F/E112K triple mutant of soybean ascorbate peroxidase
  - o Addgene construct number 42607
  - o Recommend using APEX2 instead
- APEX from pea:
  - o K14D/W41F/E112K triple mutant of pea ascorbate peroxidase
  - o Addgene construct numbers 40306, 44439, and 44440
  - o Recommend using APEX2 instead
- W41FAPX (dimeric):
  - o W41F single mutant of soybean ascorbate peroxidase
  - o Addgene construct number 42608
  - o Recommend using APEX2 instead
- W41FAPX (dimeric) from pea:
  - o W41F single mutant of pea ascorbate peroxidase
  - o Addgene construct number 40307
  - o Recommend using APEX2 instead
- Tandem dimer W41FAPX:
  - Two copies of W41F pea ascorbate peroxidase linked by a 28-amino acid linker
  - o Addgene construct number 40863

## o Recommend using APEX2 instead

## References:

- 1. Lam et. al., unpublished results.
- 2. Rhee, H.-W.; Zou, P.; Udeshi, N.D.; Martell, J.D.; Mootha, V.K.; Carr, S.A.; Ting, A.Y. Proteomic Mapping of Mitochondria in Living Cells via Spatially-Restricted Enzymatic Tagging. *Science*, **2013**, *339*, 1328-1331.
- 3. Martell, J.D.; Deerinck, T.J.; Sancak, Y.; Poulos, T.L.; Mootha, V.K.; Sosinsky, G.E.; Ellisman, M.H.; Ting, A.Y. Engineered ascorbate peroxidase as a genetically-encoded reporter for electron microscopy. *Nature Biotechnology*, **2012**, *30*, 1143-1148.