EziG™ Instruction Manual

Immobilize any His-tagged enzyme by a simple standardized procedure. Use flow chemistry or reuse your biocatalyst in batch reactions.

EnginZyme

Making biocatalysis your first choice

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Step I: Identify the most suited EziG carrier (EziG 1, 2 or 3) for your enzyme

1. Prepare cell free extract (CFE), culture supernatant or a purified preparation with His_6-enzyme in a suitable buffer
   - **20 mM phosphate or HEPES buffer, 500 mM NaCl, pH 7-8** free from cell debris, is recommended. If needed, binding selectivity may be increased by addition of 25-75 mM imidazole.

2. Quantify the active enzyme with a suitable activity assay, with an aliquot from the enzyme preparation
   - The total active enzyme mass can be calculated from the activity if the **U/g pure enzyme** is known. The estimated mass by standard protein quantification methods (e.g. Bradford) can otherwise be used. A mass figure is needed to select an appropriate carrier amount in **3.**

3. Divide the preparation into 4 equal parts, add an excess of EziG (1, 2 or 3 resp.) to individual parts, leaving one part as control
   - EziG can bind 15-40%(w/w) enzyme. For now, aim at 10% loading (0.9 g EziG per 0.1 g enzyme) or lower (use the figure obtained in **2.** for calculating the enzyme mass). If total protein (in CFE) is used, the loading will be underestimated which is not an issue at this stage.

4. Incubate the preparations for 30 min with stirring
   - Selective binding of the His-tag will occur, resulting in enriched immobilized enzyme.
   - Orbital shaker, end-over-end or propeller stirring is preferred. **Do not use magnetic stirring** since it grinds and destroys the EziG carrier.

5. Confirm the complete binding of enzyme on the 3 EziG carriers
   - After sedimentation of the EziG-enzymes, perform the assay used in **2.** with the corresponding aqueous parts to ensure that complete binding of enzyme has occurred. **No dissolved enzyme should be observed.**
   - If total protein content (in CFE) was used instead of an activity assay, recover the aqueous parts and include them in the target reaction test in **7. in which no target enzyme activity should be found**.

6. Wash the EziG-enzymes (optional)
   - Wash with the same buffer as used for the enzyme preparation in **1.** to remove native protein and other impurities. Separate by filtration, or pipetting after sedimentation.

7. Run target reactions (not an activity assay) with the 4 preparations
   - Separate the EziG-enzymes from the aqueous solutions by filtration, or pipetting after sedimentation.
   - Add the same amount of reaction mixture to the EziG-enzymes as well as the control fraction with dissolved enzyme (and aqueous parts from **5.** if total protein assays were used), compensate for differences in volume by adding buffer. **Use pH 5-10, but as high as possible within the active range of the enzyme**.
   - **Do not use magnetic stirring** since it grinds and destroys the EziG. End-over-end or propeller stirring is recommended.
   - **Do not use assay reactions with low concentrations of analyte.** The porous material will adsorb the analyte leading to underestimations of the activity. Use a minimum of 20 mM substrate, and quantify by e.g. GC or HPLC.
   - The retained activity for each carrier is found by comparing the reactions (initial rate and/or conversion after a given time) of the immobilized preparations with the non-immobilized enzyme in the control reaction. 40-100% is expected for most enzymes.

8. Test the reusability of the EziG-enzymes for the target reaction
   - Separate the EziG-enzymes from the reaction mixtures, wash according to **6.** if desired, and redo the target reactions in **7.** Iterate the procedure and measure the putative activity loss in subsequent reaction cycles. In some cases the reusability is higher with a preparation of lower retained activity, i.e. the EziG-enzyme with highest activity found in **7.** may not be the most reusable preparation. Monitor the pH in each reaction cycle, adsorption of substrates or products to the EziG carrier may cause pH changes.

✓ **Choose the most suited carrier for the enzyme based on retained activity and reusability in the chosen target reaction, then proceed to Step II.**

Step II: Find the maximum enzyme loading capacity on your EziG carrier

1. Prepare cell free extract, culture supernatant or a pure preparation with His_6-enzyme in a suitable buffer
   - **20 mM phosphate or HEPES buffer, 500 mM NaCl,** free from cell debris is recommended. If needed, binding selectivity may be increased by addition of 25-75 mM imidazole.

2. Quantify the active enzyme with a suitable activity assay, with an aliquot from the enzyme preparation
   - The total active enzyme mass can be calculated from the activity assay if the **U/g pure enzyme** is known. The estimated mass by standard protein quantification methods (e.g. Bradford) can otherwise be used. A mass figure is needed to select an appropriate carrier amount in **3.**

3. Divide enzyme preparation into 3 parts, adjust the pH to 7.5, 8.0 and 8.5 respectively, add the selected EziG, bind enzyme to carrier saturation
   - Use the best suited carrier (EziG 1, 2 or 3) found in Step I.
   - Aim at 80% loading (0.2 g EziG er 0.8 g of enzyme). This is at least a two-fold excess of enzyme.

4. Incubate the preparations for 30 min with stirring
   - Orbital shaker, end-over-end or propeller stirring is preferred. **Do not use magnetic stirring** since it grinds and destroys the EziG.

5. Confirm the presence of dissolved enzyme after immobilization to ensure saturation of the EziG carrier, and calculate the loading
   - After sedimentation of the EziG-enzymes, perform the assay used in **2.** on the aqueous parts to ensure the presence of active enzyme. **Complete binding of target enzyme should not be observed.**
   - If total protein content was used instead of an activity assay, remove the solutions and add more EziG to them according to **3.** to investigate if more enzyme can be immobilized from the solution or not. The protein quantification assay performed on the solution after this second immobilization should show a significantly lower value compared to the initial preparation in **2.**, if saturation in the first immobilization was achieved.
   - The loading can be calculated by the difference in enzyme mass in the solution, before and after immobilization.

6. Measure the catalytic activity of the EziG-enzymes by running target reactions
   - Separate the EziG-enzymes from the aqueous solutions by filtration, or pipetting after sedimentation, wash according to **6.** in **Step I** if desired.
   - Add the same amount of reaction mixture to all 3 preparations. **Use pH 5-10, but as high as possible within the active range of the enzyme**.
   - **Do not use magnetic stirring** since it grinds and destroys the EziG. End-over-end or propeller stirring is recommended.
   - **Do not use assay reactions with low concentrations of analyte.** The porous material will adsorb the analyte leading to underestimations of the activity. Use a minimum of 20 mM substrate, and quantify by e.g. GC or HPLC.
   - Compare the catalytic activity, specificity, etc. to the results from **7.** in **Step I**.

✓ **In subsequent immobilizations, use the best pH and adjust the amount of EziG to the now known loading capacity for your enzyme.**

Step III (optional): Drying and storage

1. Dry the EziG-enzymes at high vacuum in room temperature for at least 12 h, then store at 4 °C in a sealed container
   - **Vacuum is needed** to remove the water from the porous material. Prolonged drying without vacuum is not recommended.
   - In cases where vacuum drying deactivates the enzyme, store the wet preparation at 4 °C. Lyophilization can be performed, but may result in breakage of the particles and some activity loss.

Step IV: Biocatalytic reactions with EziG-enzyme

1. Use your EziG-enzyme in your batch or column reactor
   - **Do not use magnetic stirring** since it grinds and destroys the EziG.
   - After a batch reaction, filter or sediment the beads, and reuse.
   - In flow systems, you may apply aqueous and organic solvent simultaneously for *in situ* product removal.

2. Use aqueous or organic solvents
   - In aqueous environments, use pH 5-10 but as high as possible within the active range of the enzyme.
   - For water-free reactions in organic solvents, **vacuum drying is needed** to remove the water from the porous material. Prolonged drying without vacuum is not recommended.

3. Maximize reusability
   - Low pH or high concentrations of chelating compounds may increase enzyme leaching in aqueous media.
   - When the product is an acid, local pH effects may cause leaching. Use higher pH or increase buffer strength to minimize this effect.
   - Monitor the pH in each reaction cycle, adsorption of substrates or products to the EziG carrier may cause pH changes.

For support, do not hesitate to contact us:
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