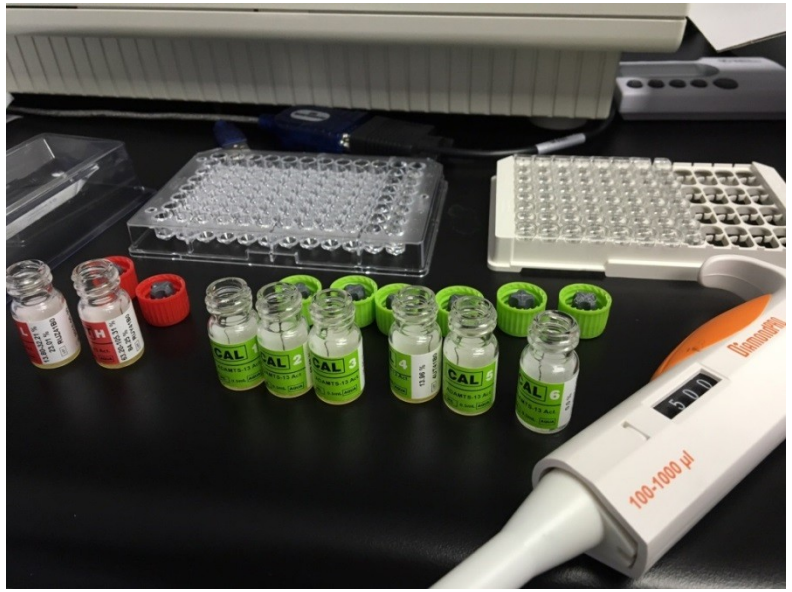
# ADAMTS-13 Activity Assay

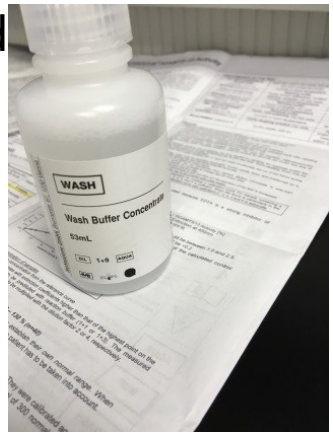
for Research Use Only in US & Canada

## Tips for Training & Validation

# Assay & Reagent Prep

- Allow all reagents to reach Room Temperature
  - **Dilute** wash buffer by mixing 1 part wash buffer with 9 parts di$H_2O$
  - **Reconstitute** vWF substrate solution with 6mL di$H_2O$.
    - 15 min after reconstitution mix for 10 sec using vortex mixer
  - **Reconstitute** calibrators and controls with 500µL di$H_2O$
    - 15 min after reconstitution mix for 10 sec using vortex mixer




# Reagent Info & Storage

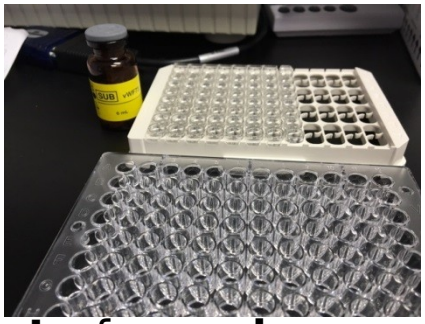
| Material/ Reagent | State | Storage | Expiration |
|---|---|---|---|
| ELISA test strips | after opening | 2-8°C in storage bag with desiccant | expiration date on bag |
| GST-vWF73 Substrate | after reconstitution | -20°C | 6 weeks |
| Calibrators, Controls | after reconstitution | -20°C | 6 months |
| Reaction Buffer | after opening | 2-8°C | 6 months |
| Conjugate | after opening | 2-8°C | 4 months |
| TMB Color Reagent | after opening | 2-8°C | expiration date on bottle |
| Wash Buffer Concentrate (10x) | after opening | 2-8°C | 6 months |
| Wash Buffer | after reconstitution | 2-8°C | 3 weeks |
| Stop Solution | after opening | 2-8°C | expiration date on bottle |

xpiration date on labels refers to storage of unopened vial at 2-8°C.
ech tip: Aliquot reconstituted calibrators, controls & substrate in no less than 250
Storage in Eppendorf vials is acceptable. Thaw at room temperature for best res

# Sample Dilution & Preparation

**te** samples and reconstituted calibrators & controls 31-fold:

sing the sample dilution microplate, pipette **5µL** of **sample**, reconstituted **calibrator** or **control**. Then add **150µL** of **reaction buffer** and mix well. **Note:** Samples, cals & controls should be tested in duplicate so this dilution will need to be done 2x for each sample.



or higher precision, use dilution tubes and add **20µL** of **sample**, reconstituted **calibrator** or **control** to the tube. Then add **600µL** of **reaction buffer** and mix well. **Note:** Samples, cals & controls should be tested in duplicate so pipette duplicate wells from this 1 dilution preparation.

**verse pipetting** is recommended.

**ubation times** begin after pipetting the last sample. *Pipetting time for e ip should not exceed 60 seconds (cals/controls/samples/conjugate).*

**mples > highest cal** should be re-tested by pre-diluting 1:2 or 1:4 with ffer. The measured concentration is then multiplied by the dilution factor

# Assay Tips

- **Reagents from kits with different lot numbers should not be used together.**
  - exclusions: wash buffer concentrate, stop solution, sample dilution microplate & plate sealers

- **Avoid using thawed calibrators, controls or substrate with fresh calibrators, controls or substrate**
  - i.e. If using thawed calibrators, all calibrators should be t

- **Precision & Performance depend on:**
  - ELISA reader: verify appropriate maintenance
  - Appropriate incubation temperature (20-25°C)
  - Appropriate incubation times – should not vary by more t
    - GST-vWF73 substrate incubation: 57-63 min
    - Sample incubation: 28.5-31.5 min
    - Conjugate reaction: 57-63 min
    - TMB Color Reagent reaction: 28.5-31.5 min
    - *Pipetting time during substrate reactions and at stopping should not exceed 10 sec per test strip.*

  Tech Tip: Incubating at the highest allowable temp (25°C) & for longest allowable time (63 or 31.5 min) will increase assay OD values.

- **Use of a multichannel pipette is encouraged.** *Remember to change tips when pipetting from row to row.*

# Limitations & Interferences

TA is a strong inhibitor of ADAMTS-13 function. **Do not use samples with EDTA**

**molysis:** No interference is observed with samples containing up to 200mg/dL emoglobin, which corresponds with a moderate haemolysis /dL.

**emia:** No interference is observed with samples containing up to 300 mg/dL Int hich corresponds with a moderate to severe concentration.

**erus:** No interference was observed with samples containing up to 15mg/dL bili njugated as well as unconjugated), which corresponds with a moderate to seve ncentration.

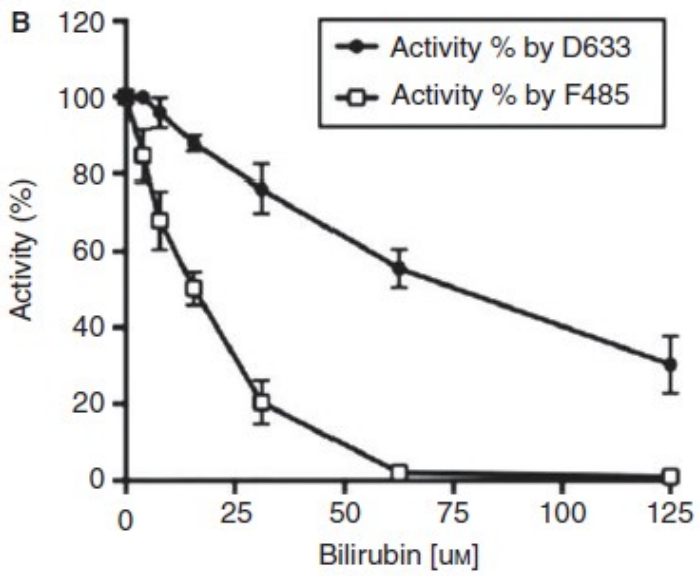
**eumatoid factor:** No interference was observed up to 30U/mL RF, with corresp th a 2-fold concentration of normal.

**ti CD20 antibodies:** No interference was observed up to a level of 200µg/mL, hich corresponds to the upper level of serum concentrations found after tuximab administration.

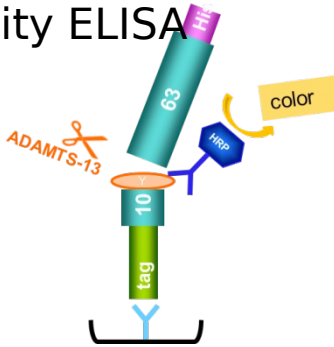
# Interferences – ADAMTS13 Assays

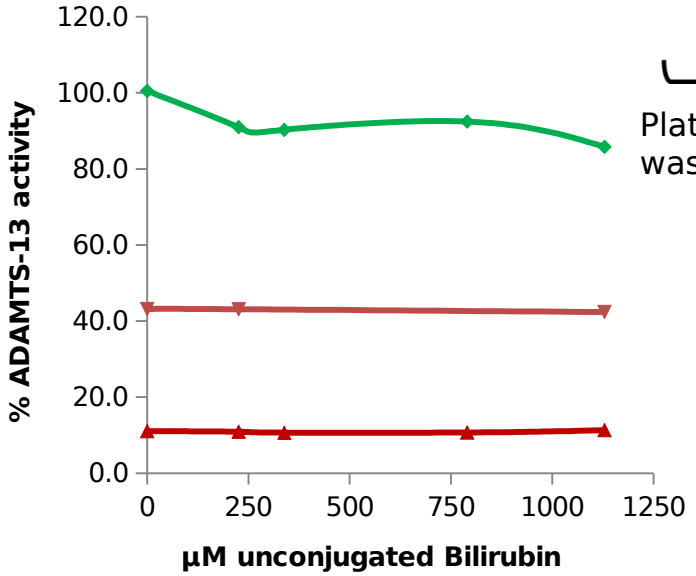
Fluorogenic Assays

**Unconjugated bilirubin interferes with fluorogenic assays:**



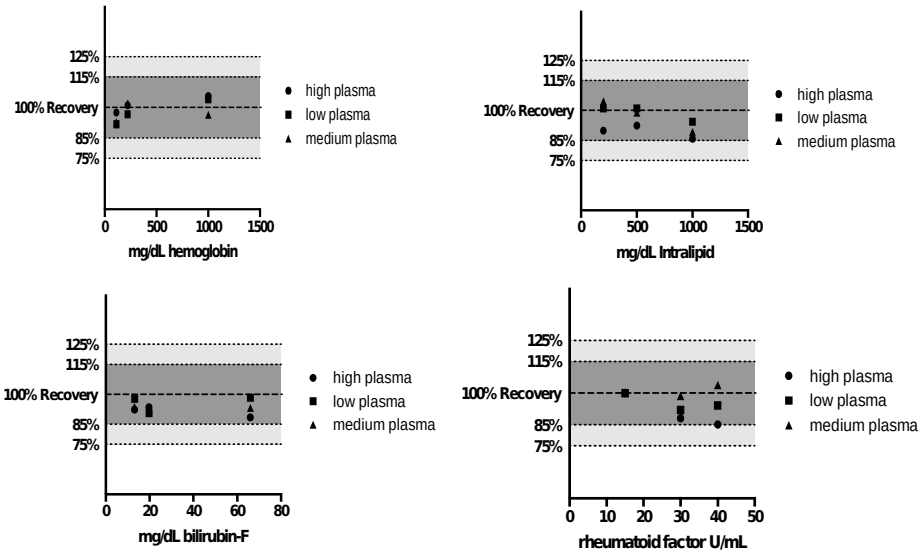

TECHNOZYM® ADAMTS-13 Activity ELISA




**No interference of unconjugated bilirubin during measurement independent of ADAMTS-13 concentration range**

# Interferences – TECHNOZYM® ADAMTS-13 Activity ELISA




**No interference of hemolysis, lipemia, conjugated bilirubin and rheumatoid factor during measurement.**

# Analysis of Results

eader settings should be established with the aid of your instrument's technical support &/or internal IT department.

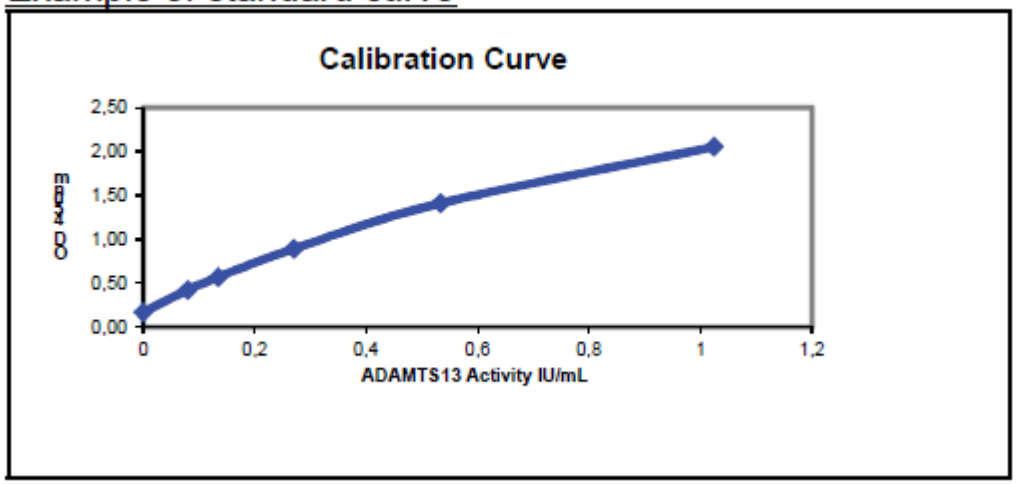
- Evaluation software (Excel spreadsheet with calculations) is available from Technoclone convert raw OD values to IU/mL , though most readers can do this as well.

et up a reference curve:

- X-axis = ADAMTS-13 activity (IU/mL)
- Y-axis = extinction at 450nm
- Graph a linear-linear plot with a best fit curve.

alidity of the test is assessed using the calculated control values.

Example of standard curve



# Lot-to-Lot Qualification Suggestions

**lot qualification procedures should always be established in conjunctio nstitution's QA policies & procedures.**

erform 20 assay runs of each control to establish your laboratory control range.

- Test using a previously qualified lot; and
- Use control range on the Certificate of Analysis from the new lot of control to accept/ reject the "test" control.

ake the mean of these 20 values to establish the mean for your laboratory.

- Use Westgard rules & a Levey-Jennings chart to accept/reject runs based on control val with the new lot.

est previously assayed samples using the new lot. Verify sample interpretation i same between lots.

# Method to Prepare Low-Activity Samples

- Heat-treat normal human plasma at **56°C** for **30 minutes** (inactivated plasma)
  - *This inactivates endogenous ADAMTS13 protease activity.*
- Heat-treated plasma can then be diluted in different concentrations (1:1, 1:2, 1:4, 1:8, 1:16) with normal plasma (pooled 5-10 individuals).
- Run the normal plasma straight to calculate expected values of diluted heat-inactivated samples & then run those as low-activity samples.

# Training Video

- www.diapharma.com/a13